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Phylogeny of European *Dolichopus* and *Gymnopternus* (Diptera: Dolichopodidae) and the significance of morphological characters inferred from molecular data

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Key words. Diptera, Dolichopodidae, *Dolichopus*, *Ethiromyia*, *Gymnopternus*, Europe, mitochondrial DNA, cytochrome oxidase I, cytochrome b, morphology, ecology, distribution

Abstract. Dolichopodidae (over 6000 described species in more than 200 genera) is one of the most speciose families of Diptera. Males of many dolichopodid species, including *Dolichopus*, feature conspicuous ornaments (Male Secondary Sexual Characters) that are used during courtship. Next to these MSSCs, every identification key to *Dolichopus* primarily uses colour characters (postocular bristles; femora) of unknown phylogenetic relevance. The phylogeny of Dolichopodidae has rarely been investigated, especially at the species level, and molecular data were hardly ever involved. We inferred phylogenetic relationships among 45 species (57 samples) of the subfamily Dolichopodinae on the basis of 32 morphological and 1415 nucleotide characters (810 for COI, 605 for Cyt-b). The monophyly of *Dolichopus* and *Gymnopternus* as well as the separate systematic position of *Ethiromyia chalybea* were supported in all analyses, confirming recent findings by other authors based purely on morphology. Within *Dolichopus*, stable species groups could be assigned to four distinct categories on the basis of their statistical support in 7 phylogenetic analyses: (i) clades significantly supported in all analyses, (ii) clades supported in trees based on DNA and combined data, but only partly in morphological trees, (iii) clades significantly supported in trees based on DNA and combined data, but not in morphological trees, and (iv) clades consistently supported only in morphological trees. The phylogeny generated here provides a better understanding of the phylogenetic relevance of some debated morphological characters used for species and species-group characterizations in the most commonly used identification keys. In this respect, postocular bristle colour proved of little phylogenetic relevance since every group with species featuring black bristles also included species with partly yellow bristles. Entirely or partly infuscated femora explained the nodes of three stable species groups and even revealed an incorrect polarity of this morphological character in three species. Four of 6 complex MSSCs and 5 of 8 more common MSSCs were found consistently in further species groups.

INTRODUCTION

The Dolichopodidae or long-legged flies are one of the most speciose families of Diptera with over 6000 described species in more than 200 genera (Grichanov, 1999a). Each year tens of new species, mainly from the Neotropics, Australasian, Oriental and eastern Palaearctic regions, are added. They occur in all terrestrial habitats, with a preference for humid sites, and although most species and highest abundances are found on muddy soil and low herbage, some species are almost entirely confined to tree trunks and other vertical surfaces (e.g. *Medetera* Fischer von Waldheim, 1819; *Sciapus* Zeller, 1842; *Neurigona* Rondani, 1857) (Pollet, 2000). Both adult and larval stages of nearly all species are assumed to be predatory on soft bodied invertebrates. Especially characteristic for this taxon are the conspicuous Male Secondary Sexual Characters (MSSC) on the legs, wings, head or abdomen which play an important role in the courtship behaviour of the males (e.g. Steyskal, 1942, 1947; Smith & Empson, 1955; Lunau, 1996; Zimmer et al., 2003).

The Dolichopodinae encompass about 25% of all described dolichopodid species, which makes it the largest subfamily. With over 8% of the known world dolichopodid species, *Dolichopus* Latreille, 1796 is by far the most species-rich genus in this fly family. Its main species diversity hotspot is located in the Holarctic Region with 317 and 134 species reported from the Nearctic Region and Europe, accounting for 24.6% and 17.0% of the respective faunas (Pollet, 2004b; Pollet et al., 2004). This pattern is also true for the eastern Palaearctic (Negrobov, 1991). The genus is entirely absent in the Neotropics as the 6 *Dolichopus* species recorded from Mexico most probably originate from the Nearctic part of the country (Robinson, 1970; Pollet et al., 2004). In con-

* MVB and MP contributed equally to this paper. MVB carried out or supervised the molecular laboratory work and was responsible for the phylogenetic analyses. MP provided the data on the morphology, ecology and European distribution of the species and investigated their relevance for the phylogeny. PIW supervises the Dolichopodidae program at the Zoological Museum. MVO obtained her Diploma in Biology with part of this project.

trast to the Afrotropical fauna with 22 *Dolichopus* species (Dyte & Smith, 1980; Grichanov, 1999b, 2004; Brooks, 2005) and the Australasian with only two (Bickel & Dyte, 1989), the Oriental region seems surprisingly rich in *Dolichopus* with several new records from China (e.g. Yang, 1996a).

Since its erection, *Gymnopternus* Loew, 1857 has always been treated as a valid genus in North America whereas European and Russian scientists considered it merely as a subgenus or synonym of *Hercostomus* (Pollet, 2004a). Recent studies by Pollet (2004a) and Brooks (2005) proved unequivocally that *Gymnopternus* is a monophyletic taxon that deserves full generic rank. Though being considerably less speciose than *Dolichopus*, *Gymnopternus* shows a similar distribution pattern with a diversity hotspot in the Nearctic (75 species, Pollet et al., 2004), 11 species in Europe (Pollet, 2004a, b; Pollet & Rampazzi, 2004) and a rich, largely unexplored fauna in the eastern Palaearctic (China, Japan) (Yang, 1996b, c, 1997a, 1997b; Yang & Saigusa, 2001a, b; Zhang et al., 2003).

Brooks & Wheeler (2005) recently erected *Ethiromyia* Brooks, 2005 as a sister clade of *Dolichopus* to include two Nearctic and one Palaearctic species, formerly placed in *Gymnopternus* (see also Pollet, 2004a). Representatives of both *Dolichopus*, *Gymnopternus* and *Ethiromyia* have a clutch of minute setae in front of the posterior spiracle that is lacking in nearly all other dolichopodine genera. In fact, this character is considered the primary synapomorphy supporting the monophyly of these three genera (Brooks, 2005).

Dolichopus is currently treated as the only valid genus name for a large number of species previously described or assigned to 11 different genera (Brooks, 2005). Indeed, successive authors have introduced structure into this taxon e.g. by erecting new genera (Lundbeck, 1912; Stackelberg, 1933) or subgenera (Frey, 1915) or dividing *Dolichopus* into sections or groups (Parent, 1938; Van Duzee, 1921). All authors (also Assis Fonseca, 1978) used the colour of the lower postocular bristles and of the femora (and their combinations) to build the basis of the intrageneric framework. Van Duzee (1921) further separated the Nearctic species into 9 Groups (A–I) purely on the basis of other colour characters whereas MSSCs were employed only at a lower taxonomic level. The use of the coloration of postocular bristles and femora for this purpose is the more surprising as lower postocular bristles in males of some species are differently coloured than in females, and the colour of the femora in some species shows a high intraspecific variability. In the introduction of the monograph on Nearctic *Dolichopus* by Van Duzee et al. (1921), also Aldrich strongly questioned the "morphological" (phylogenetic) value of the color characters. Clearly, both features are important for species diagnosis but do they also have a phylogenetic footprint?

Despite its high species richness and the presence of conspicuous morphological characters, surprisingly few authors have considered the phylogeny of Dolichopodidae, especially at the species level. In the past, attempts have been made to unravel interspecific or intergeneric relationships on the basis of mouthpart morphology (Cregan, 1941; Satô, 1991), morphology of head, thorax and abdomen, including the male genitalia (Corpus, 1989; Pollet, 1990; Maslova & Negrobov, 1996; Pollet, 1996; Pollet & Grootaert, 1998; Zhang & Yang, 2005) or even "Gesamthabitus" (Ulrich, 1980), but in most cases, cladograms were generated empirically and not based on a thorough data analysis. With his excellent study of the phylogeny in the subfamily Dolichopodinae, Brooks (2005) established a benchmark for further phylogenetic research based on morphology. He examined no less than 340 different species, 65 of which were considered representative and subsequently incorporated in his phylogenetic analysis, using 74 genital and non-genital morphological characters. This study clearly revealed the monophyly of a clade, consisting of *Dolichopus*, *Gymnopternus* and *Ethiromyia*, with e.g. *Hercostomus*, *Sybistroma* and *Poecilobothrus* belonging to a sister clade (*Ortochile* genus group). The focus of his research, however, was mainly on generic and intergeneric and much less on interspecific relationships. Molecular data have been used even less frequently. Collins & Wiegmann (2002a, b) included 5 dolichopodid species to investigate the generic and family relationships within the Empidoidea and between the Empidoidea and the lower Cyclorrhapha using 28S rDNA and elongation factor-1α (EF-1α). Unsurprisingly, this low resolution did not allow them to draw conclusions about relationships within the Dolichopodidae. Castro et al. (2002), Han et al. (2002), and Moulton & Wiegmann (2004) included sequence data from single dolichopodid species in their investigations. Masunaga (1999) combined morphological and molecular data (ITS 1 and ITS 2, unfortunately not yet available in GenBank) to investigate the relationships between marine dolichopodid species.

In the present study, we also combined morphological and molecular data, with three primary aims: (i) to unravel reliably the phylogenetic relationships between European representatives of the *Dolichopus*-*Gymnopternus*-*Ethiromyia* genus group (see Brooks, 2005); (ii) to assess the phylogenetic relevance of morphological characters of important diagnostic value that have been used in keys for almost a century; and (iii) to explore the explanatory power of ecology (habitat affinity) and zoogeographical distribution of the species in Europe with respect to the observed phylogenetic relationships.

In our study, we used two mitochondrial genes for sequencing: cytochrome oxidase subunit I gene (COI) and cytochrome b (Cyt-b). COI has been used to resolve phylogenetic relationships at many taxonomic levels, is informative across a broad range of insect taxa and its use as a standard for insect phylogenetics has been strongly advocated (Caterino et al., 2000). In particular, the terminal region of this gene is very variable in arthropods (Lunt et al., 1996; Cognato & Sperling, 2000; Martinez-Navarro et al., 2005) and therefore seems a valuable genetic marker to investigate phylogenies of entities of low taxonomic ranks such as closely-related species,

genera and subfamilies (e.g. Bernasconi et al., 2000a, b, 2001). Interestingly, a portion of about 650 nt of the COI gene has been chosen in the DNA barcode approach used for species identification (Hebert et al., 2003a, b). Cyt-b, on the other hand, is historically one of the most widely used genetic markers for phylogenetic work, particularly in vertebrates (e.g. Meyer, 1994; Johns & Avise, 1998), but also in insect phylogenetic studies (e.g. Bernasconi et al., 2001; Simmons & Weller, 2001).

Insights generated by this study may also prove applicable to other ecological, behavioural, and evolutionary projects, including those where the comparative method is central to analysis (e.g. Minder et al., 2005).

MATERIAL AND METHODS

Samples

Table 1 gives an overview of the 57 samples (specimens) and 45 species of Dolichopodinae in this study. *Dolichopus*, *Ethiromyia* and *Gymnopternus* with 39 (31 species), 1 (1 species) and 13 (9 species) samples respectively, represent the ingroup and 3 *Hercostomus* and 1 *Sybistroma* samples, each represented by 1 species, constitute the outgroup (see also Brooks, 2005). Most *Dolichopus* species included here are among the most common species of this genus in western Europe of which fresh material could readily be gathered. Further, all but one (*G. helveticus* Pollet & Rampazzi, 2004) European *Gymnopternus* species as well as the only European representative of *Ethiromyia* were incorporated in this study. Samples were exclusively gathered in western Europe (Austria, Belgium, France, Switzerland) (see Table 1 for exact locations) and were conserved in 100% alcohol (ethanol) at 4°C.

DNA extraction, amplification and sequencing

DNA extraction

DNA was extracted from flies using a Dneasy Tissue kit (Qiagen AG, Basel, Switzerland) according to the manufacturer's instructions. Whole flies were first mechanically triturated in a microtube using a TissueLyser (Mixer Mill MM 300, Qiagen AG, Basel, Switzerland). After digestion with Proteinase K (20 µg/ml), samples were applied to the columns for absorption and to wash DNA. Finally, the DNA was eluted in 200 µl of the buffer from the kit and stored at 4°C.

PCR

Standard PCR reactions were performed with 2 µl of the extracted DNA as template, 0.5 µM of each primer, 1 Unit Taq Polymerase (HotStarTaq Master Mix Kit, Qiagen AG, Basel, Switzerland) in a total volume of 50 µl (manufacturer's buffer). For both the COI and Cyt-b genes, the reaction mixtures were subjected to 15 min DNA denaturation at 94°C, 35 cycles of denaturation at 94°C for 1 min, annealing at 48–54°C for 1 min (depending on the primer combination used, see below), and elongation at 72°C for 2 min. The elongation was completed by a further 7 min step at 72°C. The PCR reactions were performed in a DNA Thermal Cycler (Perkin-Elmer Applied Biosystems, Rotkreuz, Switzerland). The amplification and sequencing primers (Microsynth GmbH, Balgach, Switzerland) are reported in Table 2. The following primer combinations were used for the (i) COI and (ii) Cyt b PCRs, respectively: (i) TL2-N-3014/C1-J-1763 (annealing at 50°C), TL2-N-3014/C1-J-2090A or 2090T (annealing at 50°C), TL2-N-3014/C1-J-2183T (annealing at 57°C) or 2183C (annealing at 54°C), and (ii) TS1-N-11683/CB-J-10933 (annealing at 48°C). The primers used are the same or modified versions of those published in Simon et al. (1994), Lunt et al. (1996), and Zhang & Hewitt (1997) and are reported in Table 2.

DNA sequencing

Templates for direct sequencing were prepared by a simple purification step of PCR products using the QIAquick PCR Purification Kit (Qiagen AG, Basel, Switzerland) following the manufacturer's instructions. Cycle sequencing reactions were performed in total volumes of 15 µl using an ABI Prism Big Dye Terminator Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems, Rotkreuz, Switzerland), purified by using DyeEx 2.0 Spin Kit (Qiagen AG, Basel, Switzerland), on an ABI Prism 3100-Avant Genetic Analyser (Perkin-Elmer Applied Biosystems), according to the manufacturer's instructions.

DNA sequence analyses

The mitochondrial sequences were handled and stored with the Lasergene program Editseq (DNAstar Inc., Madison, WI USA) and aligned separately using Megalign (DNAstar Inc); ForCon (Raes & Van de Peer, 1999), a software tool for the conversion of sequence alignments, was also used. The partition-homogeneity test (Farris et al., 1994) implemented in PAUP*4.0b10 (Swofford, 2002) was used to test whether the different datasets could be combined (COI versus Cyt-b and genetic data versus morphology). Phylogenetic reconstruction was carried out using four methods: the neighbour-joining (NJ) method, the maximum parsimony (MP) method, the maximum likelihood (ML) method, and Bayesian (BAY) analysis. The best evolutionary model of nucleotide substitution that fit the data was obtained by using the likelihood ratio test (Modeltest 3.5, Posada & Crandall, 1998). The selected model was GTR + I + G (GTR: General time reversible model, I: proportion of invariable sites, G: γ correction). The likelihood-estimated substitution rates were $R_{(A–C)} = 3.2553$, $R_{(A–G)} = 26.3839$, $R_{(A–T)} = 4.2612$, $R_{(C–G)} = 2.2906$, $R_{(C–T)} = 51.2907$, and $R_{(G–T)} = 1.0000$. The base frequencies were estimated at 0.3597 (A), 0.1751 (C), 0.0790 (G), and 0.3862 (T). The proportion of invariable sites (I) was estimated to be 0.5838, and the rate heterogeneity among variable sites was estimated to follow a gamma distribution with the shape parameter $\alpha = 0.7655$. This model of nucleotide substitution (GTR + I + G) was used for NJ and ML analyses of the combined DNA data set. MP (using the heuristic search with stepwise addition option, TBR – Tree Bisection Reconnection – branch swapping, and 100 additional replicates) and ML analyses were performed using PAUP*4.0b10, whereas both PAUP*4.0b10 and MEGA (Molecular Evolutionary Genetics Analysis version 2.1, Kumar et al., 2001) were applied for the NJ approach. Bayesian analysis was performed using MrBayes 3 (Ronquist & Huelsenbeck, 2003). The Markov chain Monte Carlo search was run with 4 chains for 1,000,000 generations, with trees being sampled every 100 generations (the first 1000 trees were discarded as "burn-in", as determined empirically). The reliability of internal branches was assessed by bootstrapping with 1000 (MP), 100 (ML) and 1000 (NJ) pseudoreplicates, while Bayesian posterior probabilities were given by the percentage of runs that produced each branch. Summarising, DNA data were analysed with NJ, MP, ML, and BAY tree reconstruction methods, while MP was applied to the morphological data, and MP and BAY to the combined data set (DNA + morphological characters).

The sequences of the two mitochondrial genes for the 57 Dolichopodidae specimens analysed in the present study have been deposited in GenBank (Table 1).

Morphological data

Table 3 shows the states of the 32 morphological characters used in this study, including 30 non-genital and 2 genital char-

TABLE 1. Dolichopodid samples and species used in this study.

Samples	Origin of specimens	GenBank Accession No.	
Species name – unique identifier	(Country*) province: locality	COI	Cyt-b
Dolichopus atripes Meigen, 1824 – 60	(BE) Limburg: Zonhoven	AY744207	AY744248
Dolichopus brevipennis Meigen, 1824 – 14	(BE) Oost-Vlaanderen: Denderhoutem	AY744186	AY744229
Dolichopus campestris Meigen, 1824 – 71	(BE) Namur: Froidfontaine	AY744212	AY744253
Dolichopus cilifemoratus Macquart, 1827 – 177	(BE) Oost-Vlaanderen: Meilegem	AY958243	AY958259
Dolichopus claviger Stannius, 1831 – 15	(BE) Oost-Vlaanderen: Denderhoutem	AY744187	AY744230
Dolichopus claviger – 53	(BE) Limburg: Zonhoven	AY744206	AY744247
Dolichopus diadema Haliday, 1832– 197	(BE) West-Vlaanderen: Knokke	AY958250	AY958265
Dolichopus excisus Loew, 1859 – 181	(BE) Oost-Vlaanderen: Meilegem	AY958245	AY958261
Dolichopus festivus Haliday, 1832 – 142	(BE) Limburg: Sint-Martens-Voeren	AY958236	AY958252
Dolichopus genicupallidus Becker, 1889 – 100	(AT) Tirol: environm. Fließ / Kaunertal	AY744183	AY744260
Dolichopus griseipennis Stannius, 1831 – 150	(BE) Limburg: Sint-Martens-Voeren	AY958237	AY958253
Dolichopus griseipennis – 186	(FR) Normandie: La Gué de la Chaine	AY958246	AY958262
Dolichopus griseipennis – 194	(BE) West-Vlaanderen: Knokke	AY958249	AY958264
Dolichopus latilimbatus Macquart, 1827 – 45	(BE) Limburg: Zonhoven	AY744200	AY744241
Dolichopus lepidus Staeger, 1842 – 48	(BE) Limburg: Zonhoven	AY744202	AY744243
Dolichopus linearis Meigen, 1824 – 157	(BE) Oost-Vlaanderen: Baasrode	AY958239	AY958255
Dolichopus longicornis Stannius, 1831 – 158	(BE) Oost-Vlaanderen: Baasrode	AY958240	AY958256
Dolichopus longitarsis Stannius, 1831 – 95	(AT) Tirol: environm. Fließ / Kaunertal	AY744218	AY744259
Dolichopus nigricornis Meigen, 1824 – 23	(BE) Oost-Vlaanderen: Neigem	AY744192	AY744234
Dolichopus nigricornis – 61	(BE) Namur: Froidfontaine	AY744208	AY744249
Dolichopus nubilus Meigen, 1824 – 180	(BE) Oost-Vlaanderen: Meilegem	AY958244	AY958260
Dolichopus pennatus Meigen, 1824 – 13	(BE) Namur: Froidfontaine	AY744185	AY744228
Dolichopus pennatus – 62	(BE) Namur: Froidfontaine	AY744209	AY744250
Dolichopus picipes Meigen, 1824 – 65	(BE) Oost-Vlaanderen: Denderhoutem	AY744211	AY744252
Dolichopus plumipes (Scopoli, 1763) – 3	(BE) Oost-Vlaanderen: Denderhoutem	AY744196	AY744227
Dolichopus popularis Wiedemann, 1817 – 2	(BE) Oost-Vlaanderen: Denderhoutem	AY744190	AY744226
Dolichopus sabinus Haliday, 1838 – 117	(BE) West-Vlaanderen: Knokke	AY744184	AY744261
Dolichopus signatus Meigen, 1824 – 46	(BE) Limburg: Zonhoven	AY744201	AY744242
Dolichopus signatus – 135	(BE) Limburg: Sint-Martens-Voeren	AY958235	AY958251
Dolichopus simplex Meigen, 1824 – 50	(BE) Limburg: Zonhoven	AY744203	AY744244
Dolichopus subpennatus Assis Fonseca, 1976 – 153	(BE) Oost-Vlaanderen: Baasrode	AY958238	AY958254
Dolichopus tanythrix Loew, 1869 – 43	(BE) Limburg: Zonhoven	AY744199	AY744240
Dolichopus trivialis Haliday, 1832 – 64	(BE) Namur: Froidfontaine	AY744210	AY744251
Dolichopus ungulatus (Linnaeus, 1758) – D3	(CH) Ticino: Castro	AY744219	AY744224
Dolichopus ungulatus – 17	(BE) Oost-Vlaanderen: Denderhoutem	AY744188	AY744231
Dolichopus ungulatus – 24	(BE) Limburg: Zonhoven	AY744193	AY744235
Dolichopus urbanus Meigen, 1824 – 1	(BE) Oost-Vlaanderen: Denderhoutem	AY744182	AY744225
Dolichopus vitripennis Meigen, 1824 – 29	(BE) Limburg: Zonhoven	AY744195	AY744237
Dolichopus wahlbergi Zetterstedt, 1843 – 76	(BE) Namur: Froidfontaine	AY744213	AY744254
Ethiromyia chalybea (Wiedemann, 1817) – 81	(BE) Oost-Vlaanderen: Denderleeuw	AY744214	AY744255
Gymnopternus aerosus (Fallén, 1823) – 25	(BE) Limburg: Zonhoven	AY744194	AY744236
Gymnopternus angustifrons (Staeger, 1842) – 52	(BE) Limburg: Zonhoven	AY744205	AY744246
Gymnopternus assimilis (Staeger, 1842) – 88	(BE) Oost-Vlaanderen: Denderleeuw	AY744216	AY744257
Gymnopternus blankaartensis (Pollet, 1990) – 90	(BE) Oost-Vlaanderen: Denderleeuw	AY744217	AY744258
Gymnopternus brevicornis (Staeger, 1842) – 36	(BE) Limburg: Zonhoven	AY744198	AY744239
Gymnopternus brevicornis – 174	(FR) Normandie: La Gué de la Chaine	AY958242	AY958258
Gymnopternus brevicornis – 190	(FR) Normandie: Vrigny	AY958247	AY958263
Gymnopternus celer (Meigen, 1824) – 18	(BE) Oost-Vlaanderen: Denderhoutem	AY744189	AY744232
Gymnopternus celer – 51	(BE) Limburg: Zonhoven	AY744204	AY744245
Gymnopternus celer – 170	(BE) Oost-Vlaanderen: Ninove	AY958241	AY958257
Gymnopternus cupreus (Fallén, 1823) – 21	(BE) Oost-Vlaanderen: Neigem	AY744191	AY744233
Gymnopternus metallicus (Stannius, 1831) – 30	(BE) Limburg: Zonhoven	AY744197	AY744238
Gymnopternus silvestris (Pollet, 1990) – 82	(BE) Oost-Vlaanderen: Denderleeuw	AY744215	AY744256
Hercostomus nanus (Macquart, 1827) – 87	(BE) Oost-Vlaanderen: Denderleeuw	AY744223	AY744262
Hercostomus nigripennis (Fallén, 1823) – 59	(BE) Limburg: Zonhoven	AY744221	AY744264
Hercostomus parvilamellatus (Macquart, 1827) – 4	(BE) Oost-Vlaanderen: Denderhoutem	AY744220	AY744263
Sybistroma obscurellum (Fallén, 1823) – 83	(BE) Oost-Vlaanderen: Denderleeuw	AY744222	AY744265

*AT – Austria; BE – Belgium; CH – Switzerland; FR – France.

TABLE 2. Amplification and sequencing primers used. Nomenclature of the primers follows the standard given by Simon et al. (1994) and also adopted by Zhang & Hewitt (1997).

Target Gene	Primer	Strand	Size (nt)	Sequence 5'-3'
COI gene	C1-J-1763	Major	24	TATAGCATTCCCACGAATAAATAA
COI gene	C1-J-2090T	Major	24	AGTTTTAGCAGGAGCAATTAC**T**AT
COI gene	C1-J-2090A	Major	24	AGTTTTAGCAGGAGCAATTAC**A**AT
COI gene	C1-J-2183T	Major	23	CAACATTTATTTTGATT**T**TTTGG
COI gene	C1-J-2183C	Major	23	CAACATTTATTTTGATT**C**TTTGG
COI gene	C1-J-2630*	Major	24	TTTATCAATAGGAGCAGTATTTGC
COI gene	TL2-N-3014	Minor	25	TCCATTGCACTAATCTGCCATATTA
Cyt-b gene	CB-J-10933	Major	26	TATGTTTTACCTTGAGGACAAATATC
Cyt-b gene	TS1-N-11683	Minor	25	AAATTCTATCTTATGTTTTCAAAAC

*Primer used for sequencing purposes only, specifically designed for this study.

acters checked exclusively in male specimens. Characters were selected on the basis of their diagnostic value in identification keys and thus comprise general colour features as well as MSSCs, except for autapomorphies (MSSCs present in a single species in the present data set). All characters were equally weighted and treated as unordered. Character polarity was based on outgroup comparison, with the most plesiomorphic state indicated by "0" and the most apomorphic state indicated by "3". The following characters were considered (I: fore leg; II: mid leg; III: hind leg; 5 tarsal segments with tarsomere 1 as most basal and tarsomere 5 as most apical):

1. Pubescence of face: (0) bare face; (1) one to multiple setae present on clypeus and/or epistoma. In some (*Gymnopternus*) species, only females show a pubescent clypeus (see Pollet, 2004a).
2. Colour of antenna: (0) entirely black; (1) mainly dark with at least part of scape and/or pedicel pale; (2) mainly pale with at least part of 1st flagellomere pale.
3. Colour of lower postocular bristles: (0) dark; (1) pale (yellow to white). In all species, the upper postocular bristles are dark brown to black.
4. Clutch of small setae in front of posterior spiracle (thorax): (0) absent; (1) present. This clutch consists of 6–10, mostly pale setae in the European *Gymnopternus* species (see Pollet, 2004a). Corresponds to character no. 15 in Brooks (2005).
5. Swelling of costal vein between humeral crossvein and vein R_1: (0) absent; (1) present. This feature is also found in females, only less distinct.
6. Swelling of costal vein at junction of vein R_1 with costal vein (costal stigma): (0) absent; (1) present. Absent in females.
7. Course of veins R_{4+5} and M_1: (0) parallel; (1) gradually converging to wing apex, vein M_1 without distinct bend; (2) weakly to strongly converging, vein M_{1+2} with smooth to distinct bend. Corresponds to character no. 34 in Brooks (2005).
8. Vein M_2: (0) present, complete; (1) present, reduced to stub; (2) absent. Corresponds to character no. 33 in Brooks (2005).
9. Wing colour: (0) transparent; (1) distinctly darkened.
10. Colour of squamal fringe: (0) entirely dark; (1) mixed dark and pale; (2) entirely pale.
11. Size of squamal fringe: (0) normal; (1) distinctly enlarged, undulating.
12. Colour of femora: (0) mainly to entirely dark; (1) mainly to entirely pale (yellow). No species included in this study had an ambiguous femoral colour.
13. Tarsus I, tarsomeres $I_{2–4}$ with regular fringe of erect, uniformly short setae on anterior or anteroventral face: (0) absent; (1) present.
14. Tarsus I, tarsomere I_5: (0) of same width as tarsomeres $I_{1–4}$; (1) enlarged and laterally compressed. Corresponds to character no. 23 in Brooks (2005).
15. Tibia I with long, curved apicoventral bristle: (0) absent; (1) present. Corresponds to character no. 20 in Brooks (2005).
16. Femur II with ventral fringe of long bristles: (0) absent; (1) present, black bristles; (2) present, yellow bristles.
17. Tarsus II, tarsomere II_1: (0) normal, without lateral leaf-like setae; (1) plumose.
18. Tarsus II, tarsomere II_1: (0) bare; (1) with one dorsal bristle.
19. Tarsus II, tarsomere $II_{2–5}$: (0) cylindrical, not flattened; (1) laterally flattened.
20. Tarsus II, tarsomeres: (0) II_5 not white; (1) $II_{3–4}$ black and II_5 white.
21. Tarsus II, whitish pilosity on tarsomeres $II_{4–5}$: (0) absent; (1) present.
22. Femur II, anterodorsal preapical bristles: (0) absent; (1) present, one bristle; (2) present, 2–3 bristles.
23. Coxa III, colour: (0) dark or metallic; (1) pale (yellowish).
24. Femur III, anterodorsal preapical bristles: (0) absent; (1) present, one bristle; (2) present, 2–3 bristles.
25. Femur III with ventral fringe of long bristles: (0) absent; (1) present, black bristles; (2) present, yellow bristles.
26. Femur III, colour of apex: (0) pale; (1) darkened. The assumed apomorphic state only applies to species with mainly pale femora.
27. Tibia III: (0) of normal width; (1) strongly swollen, either in part or entirely.
28. Tibia III, fine pilosity on posterior or posterodorsal face: (0) absent; (1) present. When present, this pilosity is always found in the basal ½ of the tibia and can be restricted to a small area or occupy the entire basal ½, with a narrow to broad extension towards the apex of the tibia. Setae that make up this pilosity are connected to internal glands that are assumed to play a role in pheromone production (see Olejníček et al., 1995; Bourandas, 1999).
29. Tibia III, apical oblique row of fine whitish setulae ("ciliolarium" sensu Steyskal, 1973): (0) absent; (1) small; (2) very large.
30. Tarsus III, tarsomere III_1: (0) bare; (1) with one dorsal bristle; (2) with 2–4 dorsal bristles; (3) with numerous dorsal bristles.
31. Hypopygium, hypandrium and epandrial lobes: (0) entirely symmetrical; (1) largely symmetrical; (2) strongly assymetrical. Corresponds roughly with character no. 64 in Brooks (2005).
32. Hypopygium, cercus: (0) simple, rather small, without strong marginal bristles; (1) *Dolichopus*-like, i.e. well developed, normally white with dark marginal border and strong apical bristles. Corresponds to character no. 68 in Brooks (2005).

These morphological characters (MC) can be divided into four categories: MC_1 comprising rather unique and complex

TABLE 3. Morphological character matrix of Dolichopodidae investigated.

Species (samples)	Morphological characters																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Ingroup																																
Dolichopus atripes (60)	0	0	1	1	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	2	1	1
Dolichopus brevipennis (14)	0	0	0	1	0	1	2	2	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	1	2	0	1	1	2	2	1	1
Dolichopus campestris (71)	0	0	0	1	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	0	0	1	2	1	1
Dolichopus cilifemoratus (177)	0	2	1	1	0	1	2	2	0	0	0	1	1	0	0	0	0	1	0	0	0	1	1	1	2	0	1	1	1	2	1	1
Dolichopus claviger (15; 53)	0	1	1	1	0	1	2	2	0	2	1	1	0	1	0	2	0	1	0	0	0	2	0	2	0	0	0	1	1	2	1	1
Dolichopus diadema (197)	0	0	1	1	0	1	2	1	0	1	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0*	1	0	1	1	2	1	1
Dolichopus excisus (181)	1	0	1	1	0	0	2	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	2	2	1
Dolichopus festivus (142)	0	2	1	1	0	1	2	2	0	0	0	1	1	0	0	0	0	1	0	0	0	1	1	1	2	0	1	1	1	2	1	1
Dolichopus genicupallidus (100)	1	0	0	1	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	2	1	1
Dolichopus griseipennis (150; 186; 194)	0	1	1	1	0	1	2	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	1	1	1	0	1
Dolichopus latilimbatus (45)	1	0	1	1	0	0	2	2	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	0	2	2	1
Dolichopus lepidus (48)	0	0	0	1	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	1	2	1	1
Dolichopus linearis (157)	0	2	1	1	0	1	2	2	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	1	2	2	1
Dolichopus longicornis (158)	0	2	1	1	0	1	2	2	0	2	0	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	1	2	2	1
Dolichopus longitarsis (95)	1	0	0	1	0	1	2	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	2	1	1
Dolichopus nigricornis (23; 61)	0	1	1	1	0	1	2	2	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	2	1	1
Dolichopus nubilus (180)	1	0	1	1	0	0	2	2	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	2	2	1
Dolichopus pennatus (13; 62)	0	1	1	1	0	0	2	2	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	1	0	0	1	1	1	2	1	1
Dolichopus picipes (65)	0	0	0	1	0	0	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2	1	1
Dolichopus plumipes (3)	0	2	1	1	0	0	2	2	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	1	2	1	1
Dolichopus popularis (2)	0	2	1	1	0	0	2	2	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	2	0	0	0	0	0	2	1	1
Dolichopus sabinus (117)	0	2	1	1	0	1	2	2	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	1	1	2	1	1
Dolichopus signatus (46; 135)	0	1	1	1	0	0	2	2	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	1	1	2	1	1
Dolichopus simplex (50)	0	2	1	1	0	0	2	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	2	1	1
Dolichopus subpennatus (153)	0	1	1	1	0	0	2	2	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	1	0	0	1	1	1	2	1	1
Dolichopus tanythrix (43)	0	0	1	1	0	1	2	2	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	1	3	1	1
Dolichopus trivialis (64)	0	2	1	1	0	1	2	2	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	2	1	1
Dolichopus ungulatus (D3; 17; 24)	0	0	0	1	0	1	2	2	0	0	1	1	0	0	0	1	0	1	0	0	0	2	0	2	1	0	0	1	1	2	1	1
Dolichopus urbanus (1)	0	2	1	1	0	0	2	2	1	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	2	1	1
Dolichopus vitripennis (29)	0	0	1	1	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	2	2	1
Dolichopus wahlbergi (76)	1	2	1	1	0	0	2	2	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	1	2	1	1
Ethiromyia chalybea (81)	0	1	0	1	0	0	0	2	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1
Gymnopternus aerosus (25)	1	0	0	1	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Gymnopternus angustifrons (52)	1	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Gymnopternus assimilis (88)	1	0	0	1	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Gymnopternus blankaartensis (90)	1	0	0	1	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Gymnopternus brevicornis (36; 174; 190)	1	0	0	1	1	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Gymnopternus celer (18; 51; 170)	1	0	0	1	1	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Gymnopternus cupreus (21)	1	0	0	1	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Gymnopternus metallicus (30)	1	0	0	1	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Gymnopternus silvestris (82)	1	0	0	1	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Outgroup																																
Hercostomus nanus (87)	0	0	0	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2	0
Hercostomus nigripennis (59)	0	0	1	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Hercostomus parvilamellatus (4)	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2	0
Sybistroma obscurellum (83)	0	0	1	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1

* Short erect pubescence present, but no fringe of strong bristles.

MSSCs present in 2–3 species (characters 13, 14, 17, 19, 20, 21); MC_2 with less specific characters that are only present in the male and can thus still be considered MSSCs (characters 6, 11, 15, 16, 25, 27, 28, 29); these characters were established in 2 to 25 *Dolichopus* species; MC_3 with male genital characters 31 and 32; and MC_4 with the remaining, non-MSSC characters. Apart from the costal stigma (character 6) in *Gymnopternus cupreus* and the apicoventral bristle of tibia I in *Ethiromyia chalybea* (character 15), derived states of characters from categories MC_1 and MC_2 are confined to *Dolichopus* species.

Ecological and distributional data

The habitat affinity of each species was determined on the basis of intensive sampling campaigns conducted between 1981 and 1997 in Belgium. By means of sweepnets, pitfall traps, coloured pan traps and Malaise traps 233,898 dolichopodid specimens were collected during this 17 year period and records were databased. In addition, both the habitat and microhabitat type of each sampling site was determined and stored, which enabled us to retrieve the habitat preference of each species in detail (see Pollet et al., 1992; Pollet, 2000).

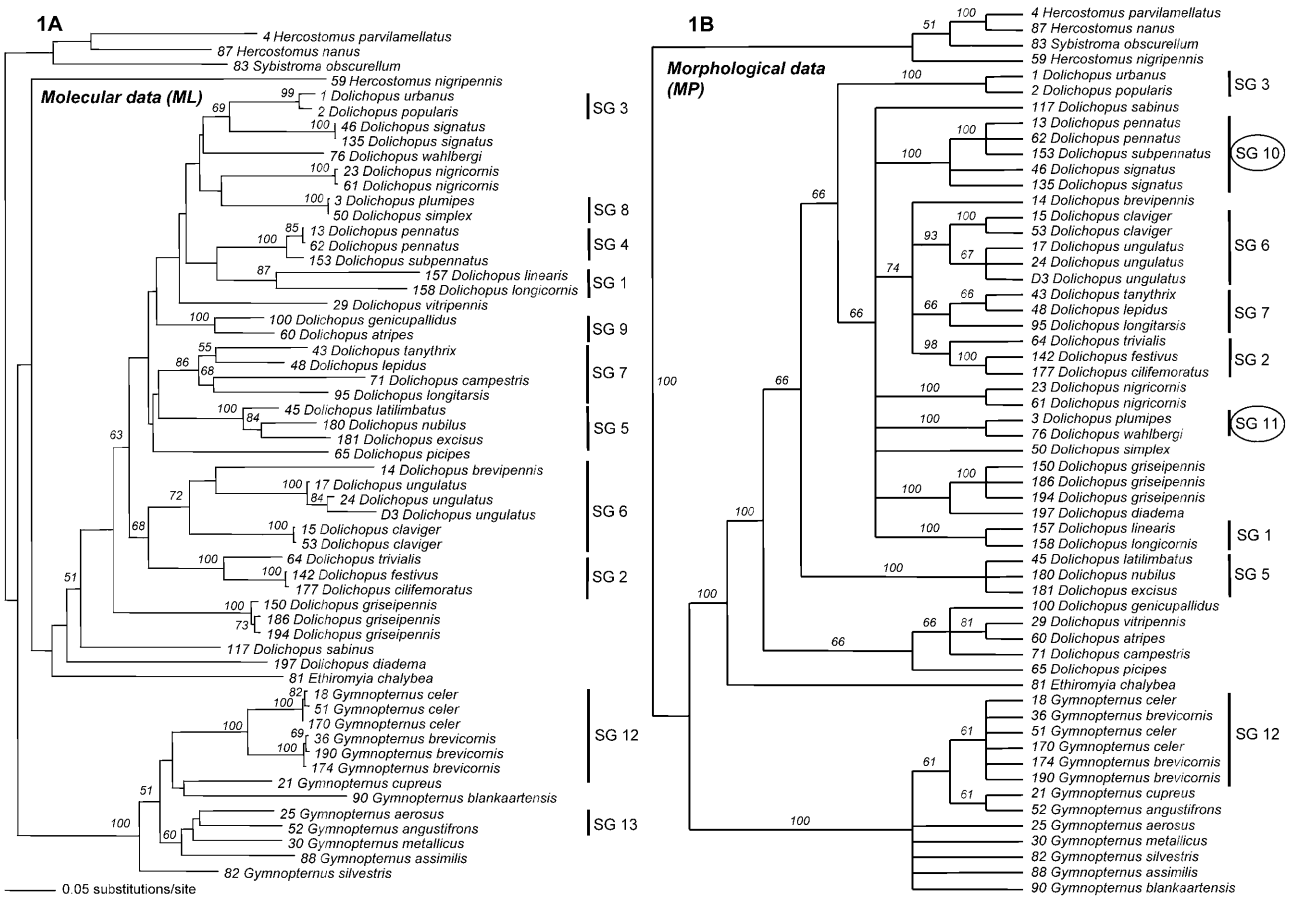

Fig. 1. A – ML tree from 100 bootstrap replicates, obtained from COI and Cyt-b combined sequences (–Ln = 15925.39419). Bootstrap support values higher than 50% are indicated above branches. B – MP 50% majority rule consensus tree of 633 equally parsimonious trees (length = 98, consistency index = 0.418, retention index = 0.820, rescaled consistency index = 0.343, homoplasy index = 0.582) obtained from 32 morphological characters. Species groups (SG) refer to Table 4; encircled SGs only supported by morphological data.

European distribution records of Dolichopodidae were compiled in the frame of the Fauna Europaea project (see Pollet, 2004b).

Abbreviations

Data sets: DNA = COI + Cyt-b sequences; Morphol = data on 32 morphological characters; Comb = combined DNA + Morphol data set. SG = Species Group. Fore, mid and hind leg indicated as leg I, II and III resp. Tarsomeres 1–5 with tarsomere 1 (= metatarsus) as most basal and tarsomere 5 as most apical. Numbers between brackets refer to morphological characters listed above.

RESULTS

Data characteristics

The total data set (Comb) of 1447 morphological and nucleotide characters was composed of 32 morphological characters and 1415 nucleotide characters (810 COI nucleotides and 605 Cyt-b nucleotides). No indels were present in the dataset. Analyses using all molecular and morphological data, involving both ingroup and outgroup species, included 570 variable sites and 502 parsimony informative sites. All 32 morphological characters were parsimony informative. When analysing the combined COI and Cyt-b dataset, 538 of 1415 nucleotide sites (38.0%) were variable and 470 (33.2%) were informative in parsimony analysis. The base composition was 31.7% A, 37.5% T, 17.6% C, and 13.2% G.

For COI, 280 of 810 nucleotide sites (34.5%) were variable within the Dolichopodinae and 250 (30.8%) informative in parsimony analysis. The base composition was 31.6% A, 37.5% T, 16.8% C, and 14.1% G. Tamura-Nei mean genetic distance (Tamura & Nei, 1993) among species of *Dolichopus* was 0.106. The intraspecific variation in *Dolichopus* was absent in *D. signatus*, 0.001 in *D. nigricornis*, 0.002 in *D. claviger*, 0.003 (range 0.001–0.005) in *D. griseipennis*, 0.004 in *D. pennatus*, and 0.024 (range 0.018–0.028) in *D. ungulatus.* Surprisingly, the genetic distance recorded between *D. plumipes* and *D. simplex*, and between *D. festivus* and *D. cilifemoratus*, was only 0.001. These results again demonstrate the potential weakness of determining species solely on the basis of genetic distances and COI sequence identities as proposed by the DNA barcoding approach. For *Gymnopternus*, Tamura-Nei mean genetic distances among species was 0.075. The intraspecific variation was 0.004 (range 0.004–0.005) in *G. celer* and 0.005 (range 0.002–0.006) in *G. brevicornis*. The mean distances between the genera considered as the ingroup were: 0.140

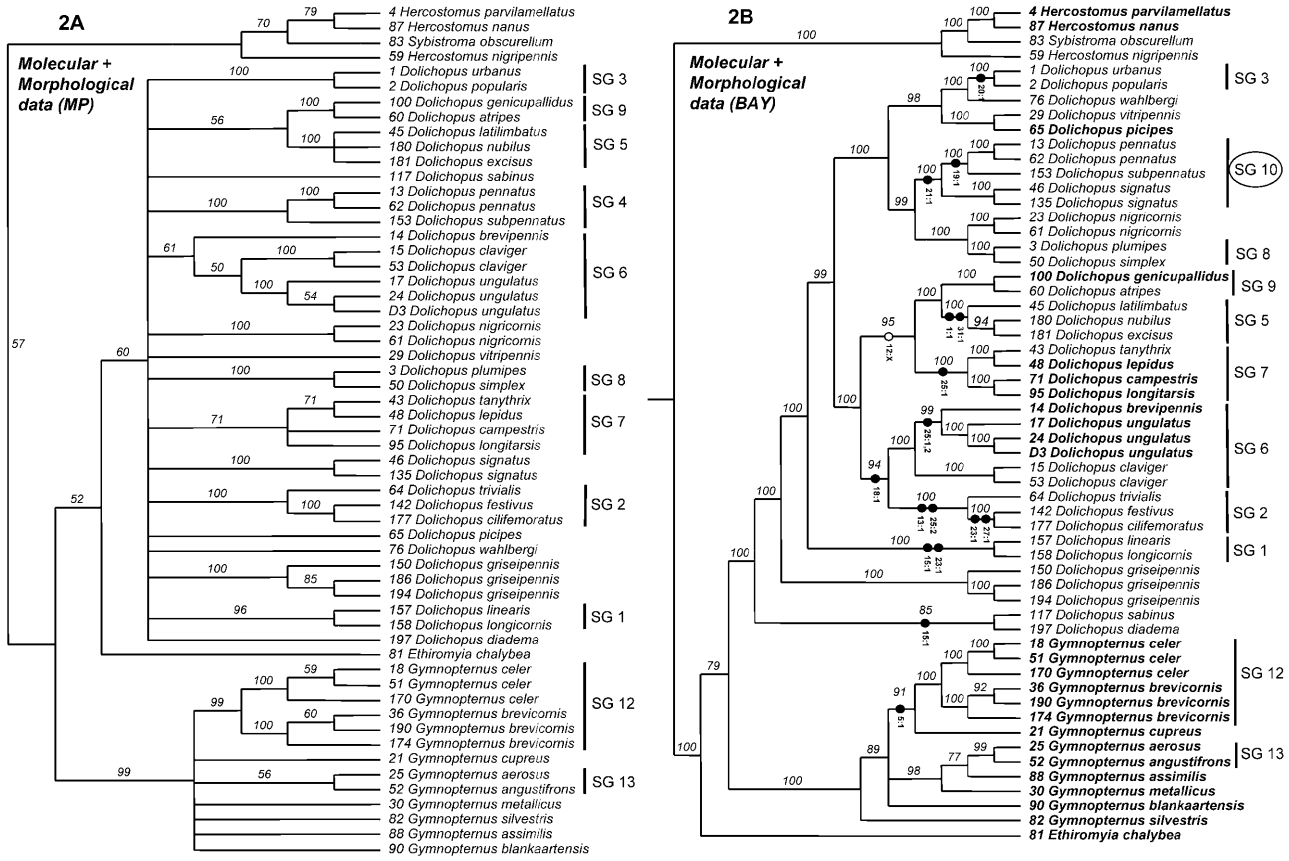

Fig. 2. A – MP 50% majority rule consensus tree from 1000 bootstrap replicates, from all molecular and morphological data combined (tree length = 3975, consistency index = 0.221; retention index = 0.498, rescaled consistency index = 0.110, homoplasy index = 0.779). Bootstrap supports over 50% are indicated above branches. B – Bayesian 50% majority rule consensus tree obtained from all molecular and morphological data combined. Values of posterior probabilities over 50% are indicated above branches. Black circles represent apomorphic character states; white circle represents plesiomorphic state of character 12 (femoral colour). Species with dark lower postocular bristles indicated in bold. Species groups (SG) refer to Table 4; encircled SG only supported by morphological data.

between *Dolichopus* and *Gymnopternus*; 0.122 between *Dolichopus* and *Ethiromyia*; and 0.128 between *Gymnopternus* and *Ethiromyia*.

For Cyt-b, 258 of 605 nucleotide sites (42.6%) were variable within the Dolichopodinae and 220 (36.3%) informative in parsimony analysis. The base composition was 31.7% A, 37.6% T, 18.7% C, and 12% G. Tamura-Nei mean genetic distance among species of *Dolichopus* was 0.139. The intraspecific variation in *Dolichopus* was absent in *D. signatus*, 0.003 in *D. pennatus* and *D. claviger*, 0.007 in *D. nigricornis*, 0.020 (range 0.019–0.022) in *D. griseipennis*, and 0.053 (range 0.017–0.076) in *D. ungulatus.* As with the COI gene, Cyt-b sequences proved to be identical in *D. plumipes* and *D. simplex* and very similar (0.007) in *D. festivus* and *D. cilifemoratus*. The Tamura-Nei mean genetic distance among *Gymnopternus* species was 0.097. The intraspecific variation was 0.003 (range 0.002–0.005) in *G. brevicornis* and 0.006 (range 0.002–0.008) in *G. celer*. The mean distances between the genera considered as the ingroup were: 0.154 between *Dolichopus* and *Gymnopternus*; 0.158 between *Dolichopus* and *Ethiromyia*; and 0.159 between *Gymnopternus* and *Ethiromyia*.

The Tamura-Nei genetic distances for both the COI and the Cyt b, and for all the samples used in this study, are presented in Tables 6 and 7.

Combined data

Molecular and morphological data were combined into one data set in an attempt to recover as reliable a phylogeny as possible. Results of the partition homogeneity test (p = 0.09 for COI vs Cyt-b, and p = 0.62 for DNA vs Morphological characters) justified this.

Phylogenetic analyses

Phylogenetic relationships as established between 57 samples of 45 dolichopodine species in the present study are presented in Figs 1 and 2, and summarised in Table 4. The molecular data set (1415 nt of the COI and Cyt-b sequences) has been analysed by NJ, MP, ML and BAY methods. MP analyses of this molecular data set, with all characters weighted equally, resulted in a single most parsimonious tree of 3509 steps (consistency index = 0.239; retention index = 0.543; rescaled consistency index = 0.130; homoplasy index = 0.761). The ML search under the GTR + I + G model of evolution is shown in Fig. 1A (–Ln likelihood = 15925.39419). The 32 morphological

TABLE 4. Overview of phylogenetic analysis results for selected, consistent nodes.

Dolichopodid species groups (SG)	Molecular data				Morphological data	Combined data	
	NJ*	ML*	MP*	BAY**	MP***	MP*	BAY**
Dolichopus							
SG1 *linearis – longicornis*	93	87	86	100	100	96	100
SG2 *cilifemoratus – festivus – trivialis*	100	100	100	100	98	100	100
SG3 *urbanus – popularis*	100	99	100	100	100	100	100
SG4 *pennatus – subpennatus*	100	100	100	100	100	100	100
SG5 *excisus – latilimbatus – nubilus*	100	100	100	100	100	100	100
SG6 *brevipennis – claviger – ungulatus*	64	72	56	100	+ (93, excl. *brev*)	61	100
SG7 *campestris – lepidus – longitarsis – tanythrix*	69	86	65	100	+ (66, excl. *camp*)	71	100
SG8 *plumipes – simplex*	100	100	100	100	–	100	100
SG9 *atripes – genucipallidus*	100	100	100	100	–	100	100
SG10 *pennatus – signatus – subpennatus*	–	–	–	–	100	–	100
SG11 *plumipes – wahlbergi*	–	–	–	–	100	–	–
Gymnopternus							
Gymnopternus clade	100	100	99	100	100	99	100
SG12 *brevicornis – celer*	100	100	99	100	61	99	100
SG13 *aerosus – angustifrons*	59	<50	61	98	–	56	99
Ethiromyia							
separated from *Gymnopternus*	+	+	+	+	+	+	+
a sister clade of *Dolichopus*	<50	<50	–	–	100	60	–

* bootstrap values; ** posterior probabilities; *** % proportion of trees showing this relationship; + item present; – item not present.

characters were analysed by MP tree reconstruction method which produced 633 equally parsimonious trees of length 98 (consistency index = 0.418; retention index = 0.820; rescaled consistency index = 0.343; homoplasy index = 0.582); Fig. 1B presents the 50% majority rule consensus tree of this analysis. Finally, the combined data set (1415 sequence data + 32 morphological characters) was analysed by MP and BAY methods. The MP analysis of all the 1447 positions, with all the characters weighted equally, generated three most parsimonious trees; Fig. 2A is the 50% majority rule consensus tree from 1000 bootstrap replicates of this analysis (tree length = 3975; consistency index = 0.221; retention index = 0.498; rescaled consistency index = 0.110; homoplasy index = 0.779).

Table 4 gives an overview of the bootstrap values and posterior probabilities supporting the phylogenetic interspecific relationship between species of *Dolichopus*, *Gymnopternus* and the position of *Ethiromyia*. From this table and from Figs 1–2, a number of conclusions on the phylogenetic relationships between genera and between species can be drawn. Below, phylogenetic hypotheses generated by DNA, morphological characters, and the combination of both data sets are compared and discussed.

The monophyly of the genus *Gymnopternus* is strongly supported by bootstrap values or posterior probabilities of ≥ 99 in all analyses.

Despite the fact that until recently, *Ethiromyia chalybea* had always been treated as *Hercostomus* or *Gymnopternus* (see Pollet, 2004a), it appears distinct from both genera in all analyses, with, however, variable statistical support. In 3 trees (DNA ML; Morphol MP; Comb MP), this taxon branches out as the sister species of the *Dolichopus* clade, whereas in two others (DNA BAY; Comb BAY) it is placed as a sister species of the *Dolichopus-Gymnopternus* group. In the DNA NJ and DNA MP dendrograms, it is positioned within the *Dolichopus* clade.

Within *Gymnopternus*, the three specimens of both *G. celer* and *G. brevicornis* are clustered in all trees, supported by bootstrap and posterior probabilities values of ≥ 99. In DNA BAY and Comb BAY, both species are clustered with *G. cupreus* (posterior probabilities of 96 and 91, respectively). All three species share a basal dilatation of the costal vein (5), but only *G. cupreus* has a conspicuous MSSC (tibia II swollen, somewhat curved and armed with 3–4 short spine-like bristles on small tubercles on the ventral surface). *G. aerosus* and *G. angustifrons* are clustered together in all analyses based on DNA and combined datasets, with variable statistical support. In the Morphol MP, however, *G. angustifrons* is invariably clustered with *G. cupreus*. The latter species both have dark femora, which is considered here a symplesiomorphy without any phylogenetic power. *G. silvestris* is positioned as the outer branch of the *Gymnopternus* clade in 4 dendrograms based on DNA and combined datasets. This pattern is not observed in the morphology-based tree.

Within *Dolichopus*, four kinds of groups of two to four species can be distinguished: Category A: clades that are supported significantly in all trees; Category B: clades that are supported in trees based on DNA and combined data, but only partly in morphological trees; Category C:

TABLE 5. Number of nodes in 4 phylogenetic trees explained by morphological characters of 4 categories (see text).

Character categories	Dendrograms			
	DNA ML	Morphol MP	DNA+Morphol MP	DNA+Morphol BAY
MC_1 (complex MSSCs)	3	5	3	4
MC_2 (more common MSSCs)	4	6	5	4
MC_3 (male genitalia)	–	–	–	–
MC_4 (non-MSSCs)	5	5	2	5
Unexplained nodes	10	–	3	11
Total nodes	22	16	13	24

clades that are supported in trees based on DNA and combined data, but not in morphological trees; and Category D: clades that are always supported in morphological trees, but not in trees purely based on DNA data.

Category A comprises 5 clades of species that are clearly morphologically related:

(i) SG1: *Dolichopus linearis* and *longicornis* share 15 synapomorphies with the apicoventral bristle on tibia I (15), the pale coxa III (23) and the strongly asymmetrical hypandrium (31) as decisive characters. The squamal fringe, considered an important diagnostic feature in Van Duzee (1921), differs in colour between the species.

(ii) SG2: *Dolichopus cilifemoratus*, *D. festivus* and *D. trivialis* have 17 synapomorphies in common, with erect minute setae on tarsomeres $I_{2–4}$ (13) and the yellow ventral fringe on femur III (25) as characters or character states unique among the species. *Dolichopus trivialis* only differs from the two other species – with very similar COI and Cyt-b sequences – in 2 morphological characters (23, 27).

(iii) SG3: *Dolichopus popularis* and *D. urbanus* are the only valid European species known with tarsomeres $II_{3–4}$ black and tarsomere II_5 contrastingly white (20). Nevertheless, these tarsomeres ($I_{3–5}$) are strongly enlarged and flattened in *D. popularis*, but of normal size in *D. urbanus*. Although both species are morphologically similar as illustrated by 12 synapomorphies, they show a number of distinct differences: *D. urbanus* has strongly infuscated wings and tibia III and a brilliantly blue thoracic dorsum, whereas *D. popularis* has transparent wings, a pale yellow tibia III and a (normal) green thorax.

(iv) SG4: *Dolichopus pennatus* and *D. subpennatus* were separated as late as 1976 (Assis Fonseca, 1976) on the basis of the shape of the posterodorsal pilosity on tibia III (28). Next to 15 other synapomorphies, both species have strongly laterally compressed tarsomeres $II_{2–5}$ (19) and a peculiar silvery white pilosity on tarsomeres $II_{4–5}$. Both synapomorphies are also observed in *D. argyrotarsis* Wahlberg, 1850 – a species not included in this analysis – whereas *D. signatus* only shares the latter character state.

(v) SG5: *Dolichopus excisus*, *D. latilimbatus* and *D. nubilus* have 12 synapomorphies in common, with the pubescence face (1), the infuscated knee of femur III (26) and the strongly asymmetrical hypandrium (31) as most distinct. No conspicuous MSSCs are found in this species group, to which *Dolichopus austriacus* Parent, 1927 and *D. andalusiacus* Strobl, 1899 also belong (both latter species not included in this study). The presence of an apicoventral bristle on tibia I of only *D. latilimbatus* that appears an important synapomorphy in SG1 is remarkable.

Category B gathers species that form moderately to strongly supported groups in trees on the basis of DNA and combined data sets. In morphological trees, however, each of these clades lack one species. The assumption that clades supported in all phylogenetic analyses purely based on DNA data can be considered reliable, renders morphological characters, present in these single species and causing their exclusion from the clade in the morphological analysis, of low phylogenetic relevance:

(i) SG6: *Dolichopus claviger*, *D. ungulatus* and *D. brevipennis*, with the two first species always clustered together. The relationship between *D. brevipennis* on the one hand and *D. claviger – D. ungulatus* on the other is not strongly supported in the DNA ML analysis either. All three species share 13 synapomorphies with only the bristle on tarsomere II_1 (18) as important. Only *D. brevipennis* and *D. ungulatus* have a strong ventral fringe on femur III (25) and black lower postocular bristles (3). Apparently, synapomorphies shared only by *D. claviger* and *D. ungulatus* (11, 16, 22, 24) have low phylogenetic value.

(ii) SG7: *Dolichopus lepidus*, *D. longitarsis*, *D. tanythrix* and *D. campestris*, with the first three species consistently forming one clade. Although they share 10 synapomorphies, there is not a single unique character. Femora in *D. longitarsis* are yellow with a black knee in femur III, whereas the other species have dark femora. *D. tanythrix* is the only species with pale lower postoculars. Morphological characters that are responsible for the exclusion of *D. campestris* in the morphological dendrograms include one apomorphy [2 preapical bristles on femur II (22)] and three plesiomorphies [absence of costal stigma (6); tibia III not swollen (27); tibia III without pilosity (28)] and prove to be of little phylogenetic value.

Category C consists of clades that are consistently and strongly supported in molecular and combined analyses but are totally lacking in the morphological ones:

(i) SG8: *Dolichopus plumipes* and *D. simplex* are genetically very similar as proved by the minute genetic distance in COI and the identical Cyt-b sequences and also morphologically they share 12 synapomorphies. However, none of the latter is unique to this species couple and *D. plumipes* shows a very distinct plumose tarsomere II_1 that is further found in *D. wahlbergi* but not

TABLE 6. COI Tamura-Nei genetic distances for the samples used in this study.

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	[23]	[24]	[25]	[26]	[27]	[28]	[29]	[30]	[31]	[32]	[33]	[34]	[35]	[36]	[37]	[38]	[39]	[40]	[41]	[42]	[43]	[44]	[45]	[46]	[47]	[48]	[49]	[50]	[51]	[52]	[53]	[54]	[55]	[56]	[57]
[1]																																																												
[2]	0.123																																																											
[3]	0.105	0.128																																																									
[4]	0.101	0.116	0.116																																																								
[5]	0.113	0.118	0.116	0.101																																																								
[6]	0.104	0.119	0.119	0.002	0.104																																																							
[7]	0.119	0.126	0.127	0.131	0.137	0.134																																																						
[8]	0.104	0.119	0.129	0.114	0.109	0.117	0.135																																																					
[9]	0.115	0.116	0.114	0.099	0.001	0.102	0.135	0.107																																																				
[10]	0.051	0.112	0.107	0.099	0.101	0.102	0.112	0.098	0.099																																																		
[11]	0.098	0.121	0.110	0.100	0.122	0.103	0.117	0.107	0.123	0.091																																																
[12]	0.097	0.122	0.110	0.099	0.123	0.102	0.117	0.107	0.125	0.089	0.004																																														
[13]	0.095	0.121	0.112	0.100	0.122	0.103	0.118	0.108	0.123	0.091	0.005	0.001																																												
[14]	0.098	0.112	0.119	0.114	0.097	0.116	0.120	0.061	0.095	0.081	0.097	0.097	0.099																																											
[15]	0.091	0.115	0.091	0.103	0.096	0.105	0.110	0.108	0.094	0.081	0.090	0.090	0.091	0.088																																										
[16]	0.107	0.122	0.134	0.118	0.112	0.121	0.133	0.118	0.113	0.106	0.109	0.111	0.112	0.113	0.115																																										
[17]	0.096	0.130	0.130	0.105	0.116	0.108	0.115	0.115	0.117	0.086	0.096	0.094	0.093	0.116	0.098	0.102																																									
[18]	0.095	0.103	0.115	0.099	0.108	0.100	0.115	0.095	0.107	0.088	0.094	0.092	0.094	0.091	0.084	0.115	0.100																																									
[19]	0.094	0.116	0.104	0.111	0.111	0.114	0.121	0.105	0.109	0.095	0.123	0.123	0.124	0.092	0.076	0.122	0.129	0.102																																									
[20]	0.097	0.105	0.116	0.100	0.110	0.102	0.116	0.096	0.108	0.089	0.095	0.094	0.095	0.093	0.085	0.117	0.101	0.001	0.103																																									
[21]	0.101	0.123	0.117	0.121	0.102	0.124	0.119	0.067	0.101	0.085	0.102	0.102	0.103	0.056	0.088	0.112	0.108	0.100	0.097	0.102																																									
[22]	0.111	0.120	0.109	0.118	0.119	0.120	0.110	0.107	0.117	0.096	0.114	0.113	0.114	0.106	0.089	0.119	0.093	0.088	0.119	0.089	0.107																																									
[23]	0.112	0.120	0.113	0.122	0.120	0.124	0.113	0.111	0.118	0.101	0.118	0.117	0.116	0.110	0.093	0.123	0.094	0.092	0.123	0.091	0.111	0.004																																									
[24]	0.108	0.110	0.128	0.108	0.114	0.109	0.121	0.110	0.112	0.087	0.112	0.112	0.114	0.105	0.087	0.121	0.107	0.091	0.107	0.092	0.103	0.116	0.120																																								
[25]	0.115	0.116	0.111	0.093	0.094	0.094	0.112	0.104	0.093	0.094	0.098	0.099	0.099	0.093	0.090	0.115	0.100	0.081	0.103	0.082	0.110	0.085	0.088	0.106																																								
[26]	0.112	0.107	0.131	0.107	0.109	0.109	0.117	0.108	0.108	0.093	0.111	0.109	0.111	0.101	0.106	0.116	0.105	0.088	0.114	0.089	0.116	0.104	0.106	0.095	0.109																																							
[27]	0.095	0.106	0.126	0.106	0.118	0.109	0.101	0.101	0.116	0.084	0.085	0.083	0.085	0.101	0.099	0.110	0.093	0.089	0.115	0.090	0.106	0.081	0.085	0.098	0.091	0.080																																							
[28]	0.093	0.110	0.123	0.107	0.112	0.110	0.099	0.115	0.110	0.085	0.082	0.082	0.084	0.100	0.087	0.114	0.097	0.100	0.120	0.101	0.110	0.108	0.112	0.098	0.100	0.095	0.090																																							
[29]	0.115	0.116	0.109	0.091	0.094	0.093	0.111	0.103	0.093	0.093	0.097	0.098	0.099	0.091	0.089	0.114	0.100	0.079	0.102	0.081	0.108	0.084	0.088	0.105	0.001	0.108	0.090	0.099																																							
[30]	0.095	0.106	0.126	0.106	0.118	0.109	0.101	0.101	0.116	0.084	0.085	0.083	0.085	0.101	0.099	0.110	0.093	0.089	0.115	0.090	0.106	0.081	0.085	0.098	0.091	0.080	0.000	0.090	0.090																																							
[31]	0.089	0.144	0.103	0.107	0.111	0.109	0.116	0.114	0.109	0.105	0.108	0.108	0.110	0.102	0.072	0.117	0.121	0.109	0.085	0.110	0.111	0.119	0.123	0.116	0.092	0.120	0.117	0.112	0.091	0.117																																							
[32]	0.103	0.122	0.113	0.114	0.121	0.115	0.107	0.103	0.119	0.100	0.107	0.106	0.107	0.110	0.090	0.115	0.094	0.088	0.128	0.089	0.110	0.019	0.020	0.113	0.091	0.098	0.071	0.105	0.090	0.071	0.122																																						
[33]	0.112	0.119	0.124	0.102	0.055	0.105	0.142	0.107	0.054	0.094	0.119	0.117	0.116	0.099	0.099	0.118	0.118	0.106	0.102	0.105	0.107	0.125	0.123	0.113	0.103	0.105	0.114	0.109	0.103	0.114	0.108	0.129																																					
[34]	0.101	0.105	0.134	0.094	0.125	0.096	0.132	0.137	0.126	0.110	0.110	0.109	0.107	0.131	0.111	0.136	0.122	0.104	0.121	0.106	0.132	0.118	0.120	0.110	0.112	0.124	0.117	0.110	0.112	0.117	0.128	0.121	0.116																																					
[35]	0.101	0.114	0.133	0.098	0.125	0.101	0.134	0.144	0.127	0.109	0.109	0.108	0.106	0.134	0.112	0.144	0.125	0.108	0.131	0.106	0.135	0.119	0.117	0.116	0.114	0.131	0.120	0.116	0.114	0.120	0.134	0.122	0.121	0.018																																					
[36]	0.095	0.105	0.122	0.088	0.116	0.091	0.124	0.129	0.118	0.107	0.114	0.112	0.111	0.129	0.097	0.135	0.114	0.102	0.115	0.103	0.125	0.112	0.113	0.112	0.105	0.118	0.112	0.124	0.105	0.112	0.122	0.115	0.112	0.028	0.025																																					
[37]	0.115	0.106	0.127	0.110	0.105	0.112	0.121	0.109	0.104	0.097	0.112	0.111	0.112	0.100	0.105	0.113	0.109	0.090	0.117	0.091	0.120	0.105	0.109	0.091	0.108	0.010	0.081	0.097	0.107	0.081	0.122	0.101	0.107	0.126	0.133	0.118																																					
[38]	0.105	0.133	0.129	0.108	0.117	0.109	0.110	0.108	0.115	0.103	0.101	0.103	0.104	0.117	0.112	0.106	0.111	0.108	0.115	0.106	0.114	0.112	0.111	0.109	0.100	0.112	0.103	0.112	0.098	0.103	0.119	0.106	0.131	0.129	0.129	0.123	0.110																																					
[39]	0.109	0.130	0.125	0.107	0.124	0.106	0.127	0.131	0.122	0.096	0.107	0.108	0.109	0.117	0.100	0.119	0.111	0.089	0.119	0.088	0.107	0.113	0.114	0.099	0.105	0.101	0.106	0.111	0.104	0.106	0.119	0.112	0.117	0.114	0.118	0.121	0.102	0.111																																					
[40]	0.126	0.131	0.136	0.127	0.128	0.127	0.123	0.129	0.126	0.114	0.107	0.107	0.108	0.115	0.110	0.137	0.129	0.117	0.120	0.118	0.124	0.125	0.129	0.113	0.109	0.111	0.116	0.094	0.107	0.116	0.133	0.124	0.114	0.142	0.146	0.132	0.109	0.139	0.139																																					
[41]	0.129	0.150	0.135	0.155	0.143	0.158	0.136	0.156	0.142	0.123	0.131	0.129	0.131	0.130	0.104	0.139	0.130	0.125	0.153	0.127	0.140	0.129	0.133	0.137	0.148	0.134	0.140	0.106	0.146	0.140	0.148	0.137	0.141	0.151	0.160	0.156	0.131	0.154	0.159	0.117																																					
[42]	0.136	0.152	0.141	0.146	0.125	0.149	0.140	0.136	0.123	0.127	0.134	0.133	0.134	0.120	0.124	0.130	0.147	0.134	0.150	0.135	0.120	0.143	0.147	0.136	0.139	0.131	0.132	0.114	0.137	0.132	0.131	0.142	0.127	0.149	0.156	0.149	0.129	0.144	0.156	0.131	0.066																																					
[43]	0.129	0.146	0.126	0.147	0.143	0.147	0.144	0.133	0.141	0.110	0.121	0.120	0.121	0.133	0.115	0.126	0.137	0.118	0.140	0.120	0.119	0.138	0.142	0.127	0.132	0.139	0.137	0.116	0.130	0.137	0.137	0.141	0.137	0.145	0.145	0.142	0.142	0.153	0.148	0.119	0.078	0.082																																					
[44]	0.136	0.161	0.143	0.160	0.145	0.160	0.139	0.151	0.147	0.127	0.134	0.132	0.134	0.152	0.127	0.159	0.147	0.144	0.168	0.145	0.153	0.138	0.142	0.151	0.152	0.149	0.144	0.119	0.151	0.144	0.164	0.141	0.151	0.155	0.158	0.156	0.147	0.171	0.162	0.150	0.099	0.119	0.106																																					
[45]	0.143	0.164	0.160	0.160	0.155	0.160	0.133	0.148	0.157	0.129	0.129	0.128	0.129	0.145	0.131	0.144	0.140	0.136	0.158	0.137	0.142	0.151	0.155	0.137	0.145	0.147	0.138	0.119	0.143	0.138	0.159	0.151	0.161	0.140	0.146	0.147	0.150	0.174	0.160	0.135	0.088	0.095	0.076	0.106																																					
[46]	0.142	0.167	0.163	0.163	0.158	0.163	0.136	0.148	0.160	0.127	0.132	0.131	0.132	0.145	0.134	0.142	0.140	0.135	0.157	0.137	0.142	0.154	0.158	0.137	0.148	0.151	0.142	0.122	0.147	0.142	0.159	0.155	0.164	0.143	0.149	0.150	0.154	0.173	0.159	0.139	0.091	0.098	0.075	0.109	0.002																																					
[47]	0.144	0.162	0.165	0.157	0.151	0.156	0.133	0.149	0.153	0.129	0.130	0.128	0.130	0.145	0.131	0.139	0.137	0.136	0.158	0.137	0.143	0.149	0.153	0.135	0.141	0.148	0.133	0.117	0.140	0.133	0.156	0.150	0.158	0.142	0.148	0.149	0.151	0.172	0.164	0.136	0.090	0.094	0.074	0.108	0.006	0.006																																					
[48]	0.138	0.151	0.155	0.157	0.149	0.157	0.132	0.152	0.147	0.117	0.134	0.133	0.134	0.134	0.123	0.139	0.143	0.136	0.150	0.137	0.135	0.157	0.161	0.127	0.147	0.136	0.142	0.126	0.146	0.142	0.145	0.159	0.149	0.160	0.170	0.168	0.136	0.161	0.153	0.132	0.082	0.080	0.086	0.110	0.053	0.053	0.054																																					
[49]	0.136	0.151	0.155	0.155	0.147	0.155	0.133	0.152	0.146	0.115	0.136	0.131	0.133	0.134	0.123	0.138	0.140	0.135	0.149	0.136	0.138	0.157	0.161	0.127	0.146	0.136	0.142	0.126	0.144	0.142	0.145	0.159	0.145	0.158	0.168	0.166	0.136	0.162	0.151	0.133	0.081	0.079	0.084	0.111	0.051	0.051	0.053	0.004																																					
[50]	0.134	0.148	0.151	0.151	0.146	0.151	0.129	0.148	0.144	0.111	0.133	0.131	0.133	0.129	0.118	0.134	0.140	0.130	0.166	0.132	0.137	0.153	0.157	0.126	0.142	0.132	0.137	0.124	0.140	0.137	0.143	0.155	0.144	0.157	0.164	0.162	0.132	0.161	0.150	0.129	0.080	0.075	0.080	0.107	0.047	0.047	0.049	0.005	0.004																																				
[51]	0.152	0.138	0.142	0.157	0.165	0.156	0.139	0.149	0.163	0.134	0.122	0.122	0.123	0.132	0.138	0.154	0.151	0.130	0.156	0.132	0.135	0.143	0.147	0.150	0.153	0.132	0.140	0.120	0.152	0.140	0.160	0.148	0.153	0.147	0.160	0.161	0.137	0.165	0.155	0.124	0.087	0.102	0.087	0.107	0.078	0.081	0.079	0.086	0.085	0.083																																			
[52]	0.118	0.142	0.122	0.134	0.131	0.137	0.128	0.132	0.129	0.101	0.107	0.105	0.107	0.123	0.098	0.128	0.122	0.118	0.142	0.119	0.123	0.133	0.138	0.128	0.129	0.125	0.126	0.108	0.127	0.126	0.131	0.129	0.131	0.134	0.139	0.146	0.125	0.152	0.133	0.117	0.061	0.071	0.071	0.095	0.075	0.078	0.076	0.074	0.073	0.069	0.082																																			
[53]	0.118	0.137	0.120	0.132	0.133	0.135	0.125	0.153	0.132	0.110	0.127	0.126	0.127	0.124	0.106	0.145	0.131	0.113	0.127	0.115	0.138	0.141	0.145	0.122	0.128	0.127	0.129	0.114	0.126	0.129	0.140	0.147	0.139	0.127	0.132	0.127	0.127	0.164	0.144	0.107	0.076	0.088	0.078	0.102	0.075	0.078	0.079	0.083	0.082	0.078	0.089	0.070																																		
[54]	0.122	0.136	0.115	0.129	0.124	0.132	0.125	0.126	0.122	0.110	0.123	0.122	0.120	0.122	0.119	0.130	0.116	0.122	0.123	0.121	0.122	0.116	0.114	0.129	0.123	0.124	0.123	0.108	0.123	0.123	0.137	0.123	0.115	0.145	0.148	0.142	0.122	0.143	0.128	0.125	0.121	0.123	0.121	0.136	0.141	0.144	0.139	0.132	0.128	0.126	0.125	0.128	0.126																																	
[55]	0.137	0.163	0.143	0.147	0.134	0.150	0.156	0.158	0.133	0.123	0.153	0.154	0.153	0.158	0.136	0.161	0.144	0.159	0.151	0.157	0.172	0.142	0.141	0.146	0.160	0.151	0.154	0.126	0.160	0.154	0.155	0.144	0.141	0.153	0.155	0.158	0.152	0.148	0.169	0.131	0.142	0.139	0.144	0.141	0.158	0.161	0.163	0.149	0.148	0.147	0.132	0.131	0.133	0.123																																	
[56]	0.107	0.143	0.118	0.139	0.139	0.142	0.158	0.129	0.137	0.109	0.128	0.126	0.125	0.130	0.117	0.133	0.136	0.124	0.124	0.123	0.131	0.129	0.128	0.135	0.140	0.133	0.142	0.115	0.140	0.142	0.129	0.132	0.130	0.151	0.150	0.149	0.136	0.156	0.149	0.131	0.136	0.140	0.116	0.139	0.143	0.143	0.145	0.148	0.145	0.143	0.144	0.129	0.130	0.099	0.138																																	
[57]	0.124	0.155	0.138	0.137	0.139	0.139	0.132	0.144	0.137	0.116	0.134	0.132	0.131	0.123	0.123	0.129	0.129	0.124	0.139	0.126	0.137	0.121	0.122	0.138	0.124	0.127	0.142	0.120	0.124	0.142	0.138	0.132	0.131	0.157	0.155	0.150	0.125	0.132	0.145	0.109	0.124	0.141	0.124	0.162	0.151	0.151	0.155	0.142	0.142	0.137	0.140	0.143	0.123	0.093	0.129	0.126

[1] # 60 - *Dolichopus atripes*
[2] # 14 - *Dolichopus brevipennis*
[3] # 71 - *Dolichopus campestris*
[4] # 15 - *Dolichopus claviger*
[5] #177 - *Dolichopus cilifemoratus*
[6] # 53 - *Dolichopus claviger*
[7] #197 - *Dolichopus diadema*
[8] #181 - *Dolichopus excisus*
[9] #142 - *Dolichopus festivus*
[10] #100 - *Dolichopus genicupallidus*
[11] #150 - *Dolichopus griseipennis*
[12] #186 - *Dolichopus griseipennis*
[13] #194 - *Dolichopus griseipennis*
[14] # 45 - *Dolichopus latilimbatus*
[15] # 48 - *Dolichopus lepidus*
[16] #157 - *Dolichopus linearis*
[17] #158 - *Dolichopus longicornis*
[18] # 23 - *Dolichopus nigricornis*
[19] # 95 - *Dolichopus longitarsis*
[20] #61 - *Dolichopus nigricornis*
[21] #180 - *Dolichopus nubilus*
[22] # 13 - *Dolichopus pennatus*
[23] # 62 - *Dolichopus pennatus*
[24] # 65 - *Dolichopus picipes*
[25] # 3 - *Dolichopus plumipes*
[26] # 2 - *Dolichopus popularis*
[27] # 46 - *Dolichopus signatus*
[28] #117 - *Dolichopus sabinus*
[29] # 50 - *Dolichopus simplex*
[30] #135 - *Dolichopus signatus*
[31] # 43 - *Dolichopus tanythrix*
[32] #153 - *Dolichopus subpennatus*
[33] # 64 - *Dolichopus trivialis*
[34] # D3 - *Dolichopus ungulatus*
[35] # 24 - *Dolichopus ungulatus*
[36] # 17 - *Dolichopus ungulatus*
[37] # 1 - *Dolichopus urbanus*
[38] # 29 - *Dolichopus vitripennis*
[39] # 76 - *Dolichopus wahlbergi*
[40] # 81 - *Ethiromyia chalybea*
[41] # 25 - *Gymnopternus aerosus*
[42] # 52 - *Gymnopternus angustifrons*
[43] # 88 - *Gymnopternus assimilis*
[44] # 90 - *Gymnopternus blankaartensis*
[45] # 36 - *Gymnopternus brevicornis*
[46] #190 - *Gymnopternus brevicornis*
[47] #174 - *Gymnopternus brevicornis*
[48] # 18 - *Gymnopternus celer*
[49] # 51 - *Gymnopternus celer*
[50] #170 - *Gymnopternus celer*
[51] # 21 - *Gymnopternus cupreus*
[52] # 30 - *Gymnopternus metallicus*
[53] # 82 - *Gymnopternus sylvestris*
[54] # 87 - *Hercostomus nanus*
[55] # 59 - *Hercostomus nigripennis*
[56] # 4 - *Hercostomus parvilamellatus*
[57] # 83 - *Sybistroma obscurellum*

TABLE 7. Cyt-b Tamura-Nei genetic distances for the samples used in this study.

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	[23]	[24]	[25]	[26]	[27]	[28]	[29]	[30]	[31]	[32]	[33]	[34]	[35]	[36]	[37]	[38]	[39]	[40]	[41]	[42]	[43]	[44]	[45]	[46]	[47]	[48]	[49]	[50]	[51]	[52]	[53]	[54]	[55]	[56]	[57]
[1]																																																												
[2]	0.153																																																											
[3]	0.164	0.175																																																									
[4]	0.149	0.112	0.155																																																								
[5]	0.135	0.150	0.182	0.140																																																								
[6]	0.149	0.111	0.151	0.003	0.144																																																							
[7]	0.140	0.173	0.158	0.172	0.151	0.176																																																							
[8]	0.148	0.176	0.174	0.145	0.156	0.145	0.191																																																						
[9]	0.133	0.148	0.180	0.136	0.007	0.140	0.149	0.154																																																					
[10]	0.079	0.126	0.145	0.125	0.125	0.123	0.157	0.135	0.123																																																				
[11]	0.148	0.154	0.149	0.129	0.104	0.133	0.138	0.156	0.104	0.131																																																		
[12]	0.149	0.159	0.158	0.137	0.107	0.141	0.149	0.154	0.107	0.139	0.022																																																	
[13]	0.146	0.155	0.150	0.131	0.093	0.135	0.141	0.157	0.093	0.131	0.020	0.019																																															
[14]	0.121	0.153	0.150	0.138	0.132	0.138	0.183	0.081	0.134	0.118	0.142	0.145	0.143																																													
[15]	0.132	0.165	0.146	0.125	0.134	0.129	0.147	0.139	0.132	0.135	0.116	0.124	0.116	0.127																																													
[16]	0.166	0.146	0.179	0.156	0.144	0.152	0.177	0.202	0.140	0.130	0.146	0.150	0.140	0.185	0.143																																												
[17]	0.167	0.189	0.198	0.158	0.145	0.158	0.200	0.197	0.141	0.151	0.150	0.152	0.146	0.178	0.162	0.119																																											
[18]	0.161	0.153	0.150	0.132	0.137	0.132	0.154	0.171	0.135	0.142	0.139	0.141	0.135	0.152	0.121	0.144	0.156																																										
[19]	0.175	0.172	0.141	0.135	0.154	0.135	0.167	0.163	0.154	0.146	0.148	0.168	0.148	0.151	0.131	0.176	0.182	0.159																																										
[20]	0.163	0.154	0.144	0.134	0.139	0.130	0.154	0.168	0.137	0.140	0.139	0.141	0.135	0.155	0.119	0.141	0.156	0.007	0.161																																										
[21]	0.145	0.166	0.147	0.140	0.129	0.140	0.181	0.075	0.127	0.115	0.133	0.136	0.130	0.066	0.118	0.168	0.163	0.159	0.137	0.162																																										
[22]	0.142	0.144	0.157	0.116	0.142	0.112	0.165	0.169	0.142	0.114	0.121	0.123	0.124	0.150	0.130	0.131	0.129	0.107	0.160	0.108	0.162																																									
[23]	0.142	0.139	0.157	0.116	0.138	0.112	0.165	0.169	0.138	0.116	0.121	0.123	0.124	0.150	0.130	0.129	0.131	0.107	0.160	0.108	0.160	0.003																																								
[24]	0.167	0.161	0.163	0.153	0.147	0.157	0.207	0.169	0.145	0.151	0.175	0.175	0.175	0.152	0.132	0.170	0.174	0.138	0.154	0.139	0.165	0.138	0.136																																								
[25]	0.129	0.162	0.170	0.156	0.158	0.156	0.145	0.172	0.162	0.133	0.144	0.152	0.142	0.140	0.155	0.139	0.146	0.127	0.162	0.122	0.169	0.122	0.122	0.180																																								
[26]	0.132	0.132	0.155	0.129	0.126	0.120	0.151	0.148	0.124	0.096	0.137	0.143	0.135	0.127	0.113	0.123	0.138	0.119	0.135	0.119	0.135	0.097	0.097	0.144	0.129																																							
[27]	0.148	0.142	0.170	0.147	0.139	0.145	0.161	0.155	0.135	0.125	0.139	0.137	0.133	0.136	0.126	0.142	0.152	0.130	0.160	0.126	0.148	0.127	0.127	0.142	0.118	0.093																																							
[28]	0.153	0.160	0.155	0.132	0.153	0.134	0.154	0.185	0.149	0.152	0.132	0.140	0.134	0.163	0.120	0.175	0.177	0.121	0.157	0.119	0.154	0.134	0.134	0.169	0.157	0.153	0.154																																							
[29]	0.129	0.162	0.170	0.156	0.158	0.156	0.145	0.172	0.162	0.133	0.144	0.152	0.142	0.140	0.155	0.139	0.146	0.127	0.162	0.122	0.169	0.122	0.122	0.180	0.000	0.129	0.118	0.157																																							
[30]	0.148	0.142	0.170	0.147	0.139	0.145	0.161	0.155	0.135	0.125	0.139	0.137	0.133	0.136	0.126	0.142	0.152	0.130	0.160	0.126	0.148	0.127	0.127	0.142	0.118	0.093	0.000	0.154	0.118																																						
[31]	0.140	0.152	0.141	0.131	0.146	0.130	0.148	0.146	0.144	0.133	0.138	0.143	0.135	0.133	0.087	0.154	0.173	0.149	0.121	0.149	0.138	0.129	0.129	0.160	0.138	0.119	0.134	0.149	0.138	0.134																																						
[32]	0.135	0.139	0.158	0.126	0.151	0.122	0.170	0.166	0.147	0.124	0.134	0.132	0.129	0.154	0.148	0.127	0.140	0.110	0.154	0.110	0.155	0.033	0.033	0.156	0.119	0.110	0.130	0.130	0.119	0.130	0.143																																					
[33]	0.140	0.130	0.157	0.127	0.082	0.127	0.142	0.166	0.079	0.121	0.136	0.141	0.117	0.129	0.133	0.151	0.164	0.135	0.147	0.128	0.131	0.119	0.119	0.146	0.150	0.112	0.135	0.127	0.150	0.135	0.151	0.125																																					
[34]	0.155	0.164	0.170	0.119	0.144	0.121	0.172	0.152	0.144	0.129	0.146	0.157	0.157	0.158	0.125	0.154	0.179	0.162	0.169	0.160	0.147	0.137	0.137	0.161	0.167	0.121	0.149	0.171	0.167	0.149	0.149	0.150	0.126																																			
[35]	0.129	0.122	0.148	0.104	0.143	0.103	0.164	0.126	0.141	0.120	0.142	0.153	0.154	0.138	0.126	0.160	0.170	0.140	0.147	0.136	0.127	0.123	0.123	0.126	0.148	0.106	0.116	0.147	0.148	0.116	0.132	0.134	0.118	0.064																																			
[36]	0.124	0.117	0.145	0.103	0.131	0.101	0.165	0.121	0.129	0.119	0.139	0.141	0.142	0.129	0.122	0.148	0.167	0.132	0.146	0.127	0.118	0.114	0.114	0.127	0.134	0.102	0.112	0.132	0.134	0.112	0.124	0.117	0.112	0.075	0.017																																			
[37]	0.141	0.142	0.155	0.128	0.132	0.128	0.154	0.145	0.130	0.108	0.141	0.147	0.143	0.140	0.128	0.132	0.150	0.120	0.136	0.120	0.139	0.109	0.107	0.128	0.119	0.034	0.102	0.154	0.119	0.102	0.124	0.120	0.117	0.129	0.111	0.105																																			
[38]	0.155	0.142	0.145	0.117	0.159	0.117	0.158	0.149	0.157	0.127	0.140	0.153	0.151	0.134	0.115	0.149	0.170	0.127	0.147	0.125	0.149	0.118	0.122	0.148	0.131	0.111	0.112	0.146	0.131	0.112	0.134	0.134	0.153	0.147	0.127	0.127	0.115																																			
[39]	0.139	0.159	0.155	0.136	0.149	0.134	0.171	0.171	0.147	0.115	0.154	0.156	0.147	0.141	0.146	0.152	0.158	0.132	0.168	0.134	0.164	0.102	0.102	0.151	0.130	0.095	0.122	0.171	0.130	0.122	0.134	0.115	0.134	0.140	0.129	0.120	0.097	0.143																																			
[40]	0.159	0.164	0.159	0.140	0.171	0.143	0.161	0.191	0.169	0.144	0.166	0.175	0.168	0.156	0.143	0.164	0.181	0.141	0.177	0.143	0.170	0.148	0.146	0.141	0.178	0.143	0.152	0.151	0.178	0.152	0.172	0.154	0.141	0.166	0.145	0.142	0.153	0.151	0.168																																			
[41]	0.168	0.164	0.154	0.133	0.158	0.133	0.178	0.145	0.154	0.159	0.132	0.134	0.130	0.145	0.127	0.166	0.164	0.142	0.174	0.142	0.133	0.149	0.147	0.165	0.184	0.142	0.156	0.144	0.184	0.156	0.147	0.147	0.159	0.159	0.143	0.124	0.154	0.142	0.160	0.166																																			
[42]	0.161	0.146	0.170	0.122	0.141	0.123	0.152	0.158	0.137	0.136	0.115	0.116	0.115	0.153	0.123	0.155	0.158	0.136	0.177	0.136	0.146	0.123	0.121	0.170	0.165	0.133	0.141	0.128	0.165	0.141	0.137	0.127	0.130	0.154	0.140	0.125	0.147	0.151	0.143	0.143	0.081																																			
[43]	0.181	0.187	0.175	0.142	0.157	0.144	0.168	0.191	0.156	0.174	0.121	0.131	0.125	0.169	0.134	0.178	0.185	0.175	0.184	0.175	0.158	0.155	0.153	0.168	0.194	0.155	0.169	0.153	0.194	0.169	0.160	0.163	0.154	0.154	0.142	0.141	0.167	0.159	0.167	0.168	0.105	0.083																																			
[44]	0.200	0.181	0.174	0.155	0.159	0.157	0.183	0.198	0.155	0.187	0.137	0.152	0.143	0.180	0.149	0.181	0.174	0.167	0.186	0.167	0.167	0.174	0.172	0.175	0.201	0.187	0.184	0.148	0.201	0.184	0.161	0.181	0.154	0.191	0.167	0.170	0.190	0.184	0.181	0.156	0.116	0.101	0.121																																			
[45]	0.183	0.177	0.160	0.151	0.162	0.151	0.147	0.180	0.158	0.155	0.136	0.147	0.138	0.175	0.156	0.176	0.194	0.157	0.160	0.155	0.177	0.153	0.153	0.194	0.179	0.152	0.188	0.148	0.179	0.188	0.145	0.155	0.140	0.174	0.155	0.139	0.162	0.179	0.159	0.171	0.107	0.103	0.120	0.124																																			
[46]	0.182	0.171	0.158	0.149	0.156	0.149	0.150	0.179	0.152	0.153	0.138	0.145	0.136	0.177	0.154	0.170	0.197	0.160	0.158	0.158	0.175	0.156	0.155	0.188	0.182	0.150	0.185	0.151	0.182	0.185	0.143	0.158	0.142	0.173	0.154	0.137	0.160	0.178	0.157	0.169	0.105	0.101	0.114	0.118	0.005																																			
[47]	0.181	0.175	0.158	0.149	0.160	0.149	0.145	0.178	0.156	0.153	0.134	0.145	0.136	0.173	0.154	0.174	0.192	0.155	0.157	0.153	0.175	0.151	0.151	0.192	0.177	0.150	0.186	0.146	0.177	0.186	0.143	0.153	0.138	0.172	0.153	0.137	0.160	0.177	0.157	0.169	0.105	0.101	0.118	0.122	0.002	0.003																																			
[48]	0.171	0.175	0.168	0.125	0.145	0.129	0.156	0.172	0.141	0.156	0.129	0.144	0.131	0.157	0.136	0.179	0.175	0.138	0.168	0.140	0.152	0.155	0.153	0.180	0.170	0.148	0.173	0.142	0.170	0.173	0.135	0.157	0.138	0.183	0.162	0.148	0.152	0.161	0.165	0.159	0.104	0.094	0.117	0.118	0.073	0.071	0.071																																		
[49]	0.178	0.175	0.168	0.119	0.143	0.123	0.156	0.175	0.139	0.158	0.131	0.146	0.133	0.159	0.133	0.179	0.169	0.140	0.170	0.142	0.147	0.153	0.151	0.177	0.173	0.142	0.166	0.146	0.173	0.166	0.133	0.160	0.141	0.174	0.159	0.145	0.141	0.163	0.156	0.159	0.104	0.090	0.116	0.111	0.029	0.077	0.077	0.008																																		
[50]	0.173	0.177	0.171	0.123	0.143	0.127	0.158	0.174	0.139	0.158	0.131	0.146	0.133	0.159	0.137	0.181	0.177	0.140	0.170	0.142	0.154	0.153	0.151	0.182	0.172	0.147	0.175	0.144	0.172	0.175	0.137	0.155	0.136	0.181	0.164	0.150	0.150	0.163	0.165	0.161	0.108	0.096	0.119	0.116	0.075	0.073	0.073	0.002	0.007																																	
[51]	0.150	0.165	0.151	0.128	0.141	0.128	0.143	0.160	0.137	0.140	0.113	0.117	0.111	0.152	0.136	0.154	0.176	0.152	0.171	0.151	0.143	0.132	0.132	0.167	0.173	0.133	0.153	0.140	0.173	0.153	0.141	0.131	0.128	0.141	0.129	0.117	0.145	0.146	0.155	0.145	0.094	0.083	0.104	0.110	0.103	0.102	0.101	0.110	0.111	0.108																															
[52]	0.169	0.172	0.163	0.127	0.139	0.127	0.157	0.158	0.135	0.150	0.116	0.126	0.117	0.165	0.129	0.164	0.189	0.148	0.185	0.147	0.149	0.156	0.156	0.171	0.183	0.154	0.149	0.144	0.183	0.149	0.161	0.170	0.142	0.155	0.140	0.140	0.161	0.159	0.171	0.143	0.099	0.091	0.110	0.111	0.123	0.117	0.122	0.112	0.105	0.114	0.105																															
[53]	0.163	0.149	0.160	0.127	0.144	0.129	0.129	0.166	0.141	0.139	0.129	0.142	0.135	0.152	0.137	0.155	0.163	0.149	0.147	0.145	0.146	0.152	0.152	0.169	0.152	0.123	0.144	0.143	0.152	0.144	0.143	0.154	0.125	0.156	0.127	0.121	0.136	0.138	0.150	0.153	0.108	0.082	0.117	0.122	0.103	0.101	0.101	0.098	0.096	0.100	0.096	0.104																															
[54]	0.182	0.177	0.183	0.165	0.189	0.169	0.162	0.194	0.193	0.189	0.158	0.160	0.156	0.174	0.164	0.197	0.200	0.176	0.182	0.180	0.176	0.181	0.181	0.189	0.187	0.179	0.201	0.159	0.187	0.201	0.154	0.185	0.183	0.206	0.184	0.172	0.183	0.177	0.186	0.165	0.162	0.138	0.167	0.151	0.161	0.159	0.159	0.155	0.152	0.157	0.132	0.170	0.138																															
[55]	0.186	0.209	0.187	0.170	0.162	0.174	0.184	0.178	0.158	0.175	0.168	0.180	0.172	0.165	0.154	0.198	0.228	0.184	0.169	0.187	0.148	0.188	0.188	0.178	0.187	0.156	0.173	0.148	0.187	0.173	0.162	0.187	0.166	0.184	0.159	0.155	0.162	0.172	0.191	0.178	0.161	0.157	0.180	0.195	0.188	0.187	0.186	0.168	0.167	0.170	0.165	0.163	0.148	0.176																															
[56]	0.178	0.180	0.218	0.176	0.180	0.180	0.170	0.204	0.176	0.174	0.187	0.197	0.190	0.197	0.188	0.199	0.221	0.188	0.182	0.184	0.201	0.185	0.185	0.189	0.183	0.163	0.191	0.168	0.183	0.191	0.174	0.189	0.168	0.179	0.166	0.163	0.167	0.176	0.179	0.160	0.183	0.172	0.188	0.203	0.182	0.181	0.180	0.172	0.170	0.174	0.154	0.170	0.140	0.136	0.176																															
[57]	0.214	0.182	0.197	0.160	0.183	0.160	0.179	0.202	0.179	0.200	0.180	0.185	0.172	0.180	0.163	0.186	0.188	0.168	0.198	0.168	0.184	0.166	0.164	0.189	0.187	0.171	0.185	0.170	0.187	0.185	0.183	0.182	0.161	0.180	0.156	0.154	0.169	0.180	0.174	0.170	0.163	0.161	0.170	0.174	0.160	0.163	0.158	0.178	0.175	0.180	0.138	0.174	0.150	0.155	0.181	0.154

[1] # 60 - *Dolichopus atripes*
[2] # 14 - *Dolichopus brevipennis*
[3] # 71 - *Dolichopus campestris*
[4] # 15 - *Dolichopus claviger*
[5] #177 - *Dolichopus cilifemoratus*
[6] # 53 - *Dolichopus claviger*
[7] #197 - *Dolichopus diadema*
[8] #181 - *Dolichopus excisus*
[9] #142 - *Dolichopus festivus*
[10] #100 - *Dolichopus genicupallidus*
[11] #150 - *Dolichopus griseipennis*
[12] #186 - *Dolichopus griseipennis*
[13] #194 - *Dolichopus griseipennis*
[14] # 45 - *Dolichopus latilimbatus*
[15] # 48 - *Dolichopus lepidus*
[16] #157 - *Dolichopus linearis*
[17] #158 - *Dolichopus longicornis*
[18] # 23 - *Dolichopus nigricornis*
[19] # 95 - *Dolichopus longitarsis*
[20] #61 - *Dolichopus nigricornis*
[21] #180 - *Dolichopus nubilus*
[22] # 13 - *Dolichopus pennatus*
[23] # 62 - *Dolichopus pennatus*
[24] # 65 - *Dolichopus picipes*
[25] # 3 - *Dolichopus plumipes*
[26] # 2 - *Dolichopus popularis*
[27] # 46 - *Dolichopus signatus*
[28] #117 - *Dolichopus sabinus*
[29] # 50 - *Dolichopus simplex*
[30] #135 - *Dolichopus signatus*
[31] # 43 - *Dolichopus tanythrix*
[32] #153 - *Dolichopus subpennatus*
[33] # 64 - *Dolichopus trivialis*
[34] # D3 - *Dolichopus ungulatus*
[35] # 24 - *Dolichopus ungulatus*
[36] # 17 - *Dolichopus ungulatus*
[37] # 1 - *Dolichopus urbanus*
[38] # 29 - *Dolichopus vitripennis*
[39] # 76 - *Dolichopus wahlbergi*
[40] # 81 - *Ethiromyia chalybea*
[41] # 25 - *Gymnopternus aerosus*
[42] # 52 - *Gymnopternus angustifrons*
[43] # 88 - *Gymnopternus assimilis*
[44] # 90 - *Gymnopternus blankaartensis*
[45] # 36 - *Gymnopternus brevicornis*
[46] #190 - *Gymnopternus brevicornis*
[47] #174 - *Gymnopternus brevicornis*
[48] # 18 - *Gymnopternus celer*
[49] # 51 - *Gymnopternus celer*
[50] #170 - *Gymnopternus celer*
[51] # 21 - *Gymnopternus cupreus*
[52] # 30 - *Gymnopternus metallicus*
[53] # 82 - *Gymnopternus sylvestris*
[54] # 87 - *Hercostomus nanus*
[55] # 59 - *Hercostomus nigripennis*
[56] # 4 - *Hercostomus parvilamellatus*
[57] # 83 - *Sybistroma obscurellum*

in *D. simplex* which has an entirely unmodified tarsomere II_1.

(ii) SG9: although *Dolichopus atripes* and *D. genucipallidus* share 9 synapomorphies, none is unique to this species group nor represents a conspicuous MSSC. Moreover, the lower postocular bristles (3) are black in *D. genucipallidus* and pale in *D. atripes*.

Category D includes groups that are only present and supported in the morphological dendrogram but absent in the molecular trees. In both cases below, clusters are based on conspicuous and complex morphological characters that cannot be interpreted as homoplasies:

(i) SG10: *Dolichopus pennatus*, *D. subpennatus* and *D. signatus*: this species group as characterized by the silvery white pilosity on the tarsomeres $II_{4–5}$ is also strongly supported in the Comb BAY dendrogram. *Dolichopus signatus* only differs in one of the 32 morphological characters from *D. pennatus* and *D. subpennatus* as it lacks the lateral compressed tarsomeres $II_{2–5}$. Nevertheless, the latter tarsomeres are distinctly black as in both other species. Despite its considerable morphological similarity, its genetic distance to *D. pennatus – D. subpennatus* is considerably larger than between both other species (mean Tamura-Nei genetic distance, Cyt-b between *D. signatus* and *D. pennatus* = 0.129, *D. signatus – D. subpennatus* = 0.131, *D. pennatus – D. subpennatus* = 0.033; COI, *D. signatus – D. pennatus* = 0.084, *D. signatus – D. subpennatus* = 0.073, *D. pennatus – D. subpennatus* = 0.020).

(ii) SG11: *Dolichopus plumipes*, *D. wahlbergi*: this relationship is not supported in any of the combined analyses. Both species share 14 synapomorphies, including the conspicuous plumose tarsomere II_1. Morphological differences between the species are only found in the pubescence of the face (1) and the coloration of the tibiae and tarsi.

To explain phylogenetic relationships on the basis of the species morphology, morphological characters were plotted on the Comb BAY dendrogram (Fig 2B). Table 5 gives an overview of the results involving all the 32 morphological characters considered. About one third of the nodes in the DNA ML and Comb BAY dendograms could be explained by single or multiple MSSCs. Of the 10 and 11 unexplained groups in both dendrograms, 7 proved identical.

DISCUSSION

The a priori choice of *Hercostomus* and *Sybistroma* species for outgroup comparison was based on the current systematic knowledge of the Dolichopodinae (Brooks, 2005). However, *H. nigripennis* tended (in some analyses, Fig. 1A) to cluster within the ingroup, while the remaining species (*H. parvilamellatus*, *H. nanus*, and *S. obscurellum*) demonstrated their suitability for outgroup comparison in all analyses. The fact that no consistent monophyly was found in *Hercostomus* is hardly a surprise since this genus is widely acknowledged as a waste basket for Dolichopodinae that do not fit the generic concept of *Dolichopus*.

The monophyly of *Dolichopus* and *Gymnopternus*, and the separate systematic position of *Ethiromyia chalybea* on the basis of both molecular, morphological and combined data perfectly match and support recent findings by Brooks (2005) on the basis of purely morphological data. In Brooks' analysis, the node comprising all three *Ethiromyia* species – indicated as "New Genus A" – was only weakly supported by 2 synapomorphies, the apicoventral bristle of tibia I (15) and the *Dolichopus*-like cercus (32). Both characters separate *Ethiromyia* from *Gymnopternus*, but are widespread among *Dolichopus* species. In addition, Pollet (2004a) listed many more synapomorphies that support the *Ethiromyia* clade. It is also interesting (and reassuring) to notice that though Brooks (2005) based his conclusions on the monophyly of *Gymnopternus* on two morphological, genital characters not considered in the present study, both phylogenetic analyses yielded an identical result.

With respect to its relevance for the phylogeny in *Dolichopus*, neither species with entirely dark postoculars nor those with pale lower postoculars seem to be monophyletic in any of the phylogenetic analyses. In fact, each of the three clades (*D. atripes – D. genucipallidus*; *D. brevipennis – D. ungulatus – D. claviger*; *D. tanythrix – D. lepidus – D. campestris – D. longitarsis*) with species with entirely dark postocular bristles also contains one species with pale lower postoculars. The moderate to strong support of these clades in all molecular and combined analyses seems to indicate the swift transformation of this character during speciation processes.

Of the 7 species with dark femora, only *D. vitripennis* and *D. picipes* do not form stable relationships with other species. In contrast, *D. atripes* and *D. genucipallidus* cluster together in all molecular and combined analyses, which is also true for *D. tanythrix*, *D. lepidus*, and *D. campestris* which form a relatively well-supported clade with *D. longitarsis*. Remarkably, in the DNA ML and Comb BAY trees the latter clade is extended with another stable species group (*D. excisus*, *D. latilimbatus*, *D. nubilus*) having femur III with an infuscated knee similar to *D. longitarsis*. This clade (in the Comb BAY tree) thus contains 5 species with dark femora and 4 species with only a partially darkened femur III. As this colour character is the only one that explains this node, the apical infuscation of femur III might be interpreted as an intermediate character state between entirely dark and entirely yellow and not as a synapomorphy as in the present study (26). The fact that these species are grouped together despite a possible incorrect polarization of this morphological character represents additional support for this group. Femoral colour thus does seem to be of phylogenetic value.

The presence of a dorsal bristle on tarsomere II_1 (18) is also of special phylogenetic interest. This is not only the single character that is shared by all 6 species (*D. brevipennis*, *D. ungulatus*, *D. claviger*, *D. trivialis*, *D. festivus*, and *D. cilifemoratus*) in a clade that is moderately (DNA ML: bootstrap value of 68%) or strongly supported (Comb BAY: posterior probabilities of 94), but it is also

lacking in all other *Dolichopus* species treated here. Its usefulness is further illustrated by the observation that the clade includes species with dark and pale lower postoculars and species with antennae that vary in colour from mainly pale to entirely black.

In contrast to the other complex MSSCs of category MC_1, laterally flattened tarsomeres I_5 (14) and a plumose tarsomere II_1 (17) do not seem to be phylogenetically relevant. Indeed, species featuring these two MSSCs did not form a separate clade in any of the analyses. Considering the great diversity of the first character in Nearctic species (Van Duzee, 1921), parallel evolution in this character is quite likely. Including Nearctic *Dolichopus* species in our study should provide more insight in this respect. Parallel evolution, however, is much less plausible for character 17 which is only present in five Palaearctic species (*D. plumipes*; *D. wahlbergi*; *D. parvicaudatus* Zetterstedt, 1843; *D. pectinitarsis* Stenhammar, 1852; *D. polleti* Meuffels & Grootaert, 1989). Moreover, in contrast to character 14, the fine structure of the plumosity of tarsomere II_1 is almost identical in the three species that could be examined (*D. plumipes*, *D. wahlbergi*, *D. polleti*). The observation that the compositions of COI and Cyt-b of *D. plumipes* and *D. wahlbergi* show considerable differences (Tamura-Nei genetic distance, between *D. plumipes* and *D. wahlbergi* = 0.129 for Cyt-b and 0.105 for COI, respectively) whereas they are nearly identical in *D. plumipes* and *D. simplex* (0 for Cyt-b and 0.001 for COI), a species without a plumose tarsomere II_1, is very surprising and needs further research. In this context, efforts are being made to include the extremely rare European *D. polleti* as well, as this will most certainly contribute considerably to the resolution of the analysis.

Of the MSSCs of category MC_2, neither the costal stigma (6), the pilosity (28) nor the ciliolarium on tibia III (29) explained any of the nodes. The other 6 characters support some nodes, but were not sufficiently decisive to cluster all species sharing one of these MSSCs. In Comb MP, a large squamal fringe (11) and a ventral fringe on femur II (16) explained the node with *D. ungulatus* and *D. claviger* despite numerous morphological differences between both species (2, 3, 10, 14, 25). They do show a very similar cercus shape, a character not included in this study due to its high diversity. A clade of these species with *D. brevipennis*, as present in all DNA and Comb dendrograms, is not supported by any single morphological character. Actually, it was expected that *D. nigricornis* would rather be related to *D. claviger* as both share pale lower postoculars (3), pale femora (12), a costal stigma (6), a flattened tarsomere I_5 (14), femur III with a ventral fringe (25) and a ciliolarium on tibia III (29). However, all of these characters are considered apomorphies, apparently without any phylogenetic value, as even revealed by purely morphological phylogenetic analysis. A possible explanation for the lack of phylogenetic power might be due to the low resolution used in defining characters 6, 28 and 29. Indeed, not only is a considerable diversity of these characters observed within *Dolichopus*, but each has been associated with glands with a role in courtship behaviour (Smirnov, 1948; Olejníček et al., 1995; Bourandas, 1999). A more detailed morphological and anatomical examination of these characters (including glandular structures) might contribute considerably to the unravelling of phylogenetic relationships between these *Dolichopus* species.

Ecologically, no overall pattern was observed in *Dolichopus*. Five of the 9 stable groups (see Table 4) contained species with distinctly different habitat affinities. *D. pennatus* is typical of humid forests and wooded eutrophic marshlands, *D. subpennatus* is mainly found in open habitats like humid eutrophic marshlands and on riverbanks, and *D. signatus* shows a preference for rather dry to humid heathlands and mesotrophic to oligotrophic forests.

The eurytopic *D. plumipes* is among the most common dolichopodid species in western Europe (Pollet, 2000) and occurs in all kinds of open, mesotropic to eutrophic, rather humid to humid habitats like marshlands and grasslands. In contrast, *D. simplex* is restricted to distinctly nutrient-poorer conditions like peatmoors and heathlands whereas *D. wahlbergi* is typical for rather humid, dark deciduous forests.

Also in the clade *D. brevipennis – D. ungulatus – D. claviger*, habitat preferences are very different. Like *D. plumipes*, *D. brevipennis* prefers marshlands and humid grasslands. *D. ungulatus*, on the other hand, is extremely eurytopic and common but is found in highest abundances on the banks of pools or in muddy sites in deciduous forests. *D. claviger* is a forest-inhabiting species as well, however, with its main distribution in dark, rather dry deciduous forests, even in coastal regions.

In the clade *D. campestris – D. lepidus – D. tanythrix – D. longitarsis*, only *D. campestris* is found beyond oligotrophic habitats, in rather open, short grazed, riparian sites at both stagnant and running water bodies. *D. lepidus* and *D. tanythrix* are characteristic for humid heathlands and peatbogs, although the latter seems less common but more abundant where it occurs. Finally, *D. longitarsis* prefers oligotrophic habits as well but, in contrast to the former two species, is found in highest numbers in humid, wooded sites.

D. atripes is also mainly found in humid to dry heathlands from sea level to high altitude, whereas the related *D. genucipallidus* is clearly confined to mountain habitats.

Although species in the remaining 4 species groups prefer similar habitat types, they are rarely encountered together in the field and/or show a considerably different rarity. The more common *D. popularis* and the rarer *D. urbanus* inhabit humid forests. However, the first occurs on both loamy and sandy soils while the second is restricted to slope forests and carrs with undergrowth of *Filipendula ulmaria*, mainly on loamy soils. The eurytopic *D. longicornis* is much more common than the related *D. linearis* and occurs in all kinds of open habitats with well developed vegetations. It is especially common in reedmarshes, which seems to be the preferred habitat

of *D. linearis*. *D. trivialis*, *D. festivus* and *D. cilifemoratus* are dwellers of open deciduous forest habitats and wooded marshlands. *D. trivialis* is the most common and prefers rather dry to dry forest edges, whereas *D. festivus* and *D. cilifemoratus* are considerably more hygrophilous. *D. cilifemoratus* is by far the rarest species with its main distribution in mesotrophic, wooded marshlands on loamy soils.

Finally, all species of the clade *D. excisus – D. nubilus – D. latilimbatus* are characteristic of riparian habitats of mesotrophic to eutrophic ponds and associated marshlands. Although they are often found associated in the field, small differences are observed between the species: *D. nubilus* is the most common and occurs in the widest range of habitats with a preference for reed marshes. *D. latilimbatus* is typical for wooded marshlands and carrs, whereas the rarer *D. excisus* reaches it highest abundances in open, short-grazed marshlands. *D. sabinus* and *D. diadema* which are only clustered together in the Comb BAY cladogram (with a posterior probability of 85%) are strictly halophilous species from saltmarshes.

Species forming the two stable clades in *Gymnopternus* are similar in habitat affinities with slight differences. *G. brevicornis* and *G. celer* are primarily forest-inhabiting species, with the first species preferring mature beech forests on loamy soils whereas the second mainly occurs in deciduous forests on sandy soils, and on riverbanks. On the other hand, *G. aerosus* and *G. angustifrons* reach their highest abundances in oligotrophic situations like humid heathland. While *G. aerosus* is considerably more common and more eurytopic than *G. angustifrons*, the latter is restricted to moorlands and humid heathlands (Pollet et al., 1992).

Similar to habitat preferences, the zoogeographical distributions of the species in Europe proved of little explanatory value for the observed clades. This was mainly because nearly all species are widespread. As compared to their congeners, only four species appeared more restricted: *D. brevipennis* and *D. tanythrix* are absent from central and southeastern Europe, whereas the latter species and *D. excisus* are much rarer in northern Europe (including the British Isles) as well. *D. genucipallidus* proves to be a typical mountain species with a distribution confined to France, Germany, Italy, Switzerland and Austria.

In conclusion, this study represents one of the few existing phylogenetic analyses based on both molecular and morphological data of members of the dipteran family Dolichopodidae in general, and is the first to deal in detail with members of the subfamily Dolichopodinae at a species level. The phylogenetic hypotheses provided here allowed us to clarify previous assumptions and speculations concerning the phylogenetic and systematic placement and ranking of some key taxa. Moreover, it gives us a better understanding of the phylogenetic suitability of some debated morphological characters used for species and species-groups characterization in the most commonly-used identification keys. The phylogenetic impact of MSSCs such as the flattened tarsomeres I_5, the plumose tarsomere II_1, the costal stigma, and the pilosity and ciliolarium on tibia III, however, remain unresolved. Not only could a more detailed, perhaps even anatomical, examination of some of these structures yield new insights, but the incorporation of more species, in particular of the morphologically highly diverse Nearctic *Dolichopus*, might provide a better resolution in this respect.

The phylogenetic framework generated in this study will be important for future projects on these flies, particularly for those in which the comparative method is used. Studies using the comparative method need to be based on an accurate and reliable phylogeny (see e.g. Quicke, 1993). Some Dolichopodidae species also have the potential to become suitable model organisms in other areas of biological research (e.g. behaviour and animal ecology).

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